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THE LIFE CYCLES OF TWO NEW SPECIES OF HETEROPHYIDAE, PARASITIC IN MAMMALS AND BIRDS*

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The two digenetic trematodes which form the basis of this study belong to the family Heterophyidae. The first member of this family to be described was discovered by Bilharz on April 26, 1851, in the body of a small Egyptian boy which had come to autopsy in Cairo. The parasites were found in large numbers attached to the small intestine, appearing as small red punctate objects, and on being examined under the microscope were found to be distomes. Bilharz also appears to have found the flukes a second time (v. Siebold, 1852). A brief description of the worms was included in a communication from Bilharz to von Siebold who published the data and on the basis of Bilharz's description named the worm *Distomum heterophyes*. The generic name Heterophyes was established by Cobbold in 1866 but the type species *H. heterophyes* was not carefully described until Looss's study appeared in 1894. Looss states that up to the time he started to study the problem in Egypt there appeared to be only one other record of the finding of this worm. In Alexandria he found the worms and in Cairo both the worms and the eggs not infrequently, and came to the conclusion that with a little search they would be found in a relatively large percentage of the Egyptian population.

The family Heterophyidae was established by Odhner in 1914 to include in addition to the type genus Heterophyes, the following genera: Centrocestus Looss 1899, Ascocotyle Looss 1899, Pygidiopsis Looss 1907, Apophallus Lühe 1909, Tocotrema Looss 1899 (syn. of Cryptocotyle Lühe 1899), and Scophanocephalus Jägerskiöld 1903, as well as

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the doubtful species *Distomum squamulum* Rud. 1819, *D. planicolle* Rud. 1819, and *D. trifolium* Braun 1901. Since then there have been described the following genera which have been included in this family: Metagonimum Katsurada 1912, Paracoenogonimus Katsurada 1914, Rossicotrema Skrjabin 1919, and Cotylophallus Ransom 1920.

The first species of this group to be found in the Orient was *Metagonimus yokogawai* *, found in man for the first time in 1911 by Yokogawa (Formosa), after experimental infection of dogs and cats (1911) with cysts from infected trout (*Plectoglossus altivelis*); also described from the dog (Japan) as *Loxotrema ovatum* by Kobayashi in 1912.

Leiper (1913) drew attention to the fact that *Heterophyes heterophyes* was present in China and Japan as determined by the presence of these flukes in Chinese and Japanese patients in the Albert Dock Hospital, London. He also listed *Heterophyes heterophyes, Yokogawa yokogawai* (i. e., *Metagonimus yokogawai*) and *Centrocestus cuspidatus* from the dog from Formosa after examination of material collected by Yokogawa. In 1915 Onji and Nishio described what they believed to be a second species of Heterophyes (*H. nocens*) from human cases in Japan (communicated in English by Cort and Yokogawa in 1922). In 1920 one of us (Faust) described a new species from the intestine of the monkey-eating eagle under the name *Phagicola pithecophagicola,* a fluke which on restudy has been found to belong to the genus Ascocotyle

* Leiper (1922: 364-365) refers *Metagonimus yokogawai* to *Loxotrema ovatum,* which he states was "named in 1908 by Kobayashi . . . and found by him experimentally after feeding animals upon fish containing encysted cercariae." Through the kindness of Dr. Kobayashi we have been placed in possession of the facts in the case and have also had the opportunity of examining cotype specimens of Koboyashi's species. The material is, indeed, the same as that described by Yokogawa. However, there are two difficulties in the way of accepting the name *Loxotrema ovatum* pro *Metagonimus yokogawai.* In the first place Dr. Kobayashi writes: "I have no publication on the subject during 1908. I published a preliminary report of a new genus, *Loxotrema,* in the Saikingaku Zasshi (Jour. Bacteriology) no. 204 (Oct. 10, 1912), which antedates by twenty days the publication on *Metagonimus* in the Okayama Igakkai Zasshi (Jour. Okayama Med. Assn.), no. 273 (Oct. 31, 1912)." However, Kobayashi's report does not antedate the presentation of Yokogawa's discovery by Dr. Katsurada before the Japanese Pathological Association, when the name *Heterophyes yokogawai* was assigned to Yokogawa's species, nor to the publication of this report in the Journal of the Okayama Medical Association, May 31, 1912, and June 30, 1912. Moreover, Kobayashi (l. c. 1912) recognized the validity of the species "*yokogawai*" and assigned the name *Loxotrema ovatum* to his species "because the sizes of the suckers and the genital pores were different" in the two species. Even if Kobayashi's publication had antedated the first published record of Yokogawa's discovery, the name *Loxotrema* would not be available, since it is preoccupied (MOLLUSCA Gabb 1868), in which case the species would necessarily have to be designated as *Metagonimus ovatus* Kobayashi 1912. However, the previous designation of Yokogawa's species (Katsurada 1912) leaves no room for doubt: the correct name is *Metagonimus yokogawai.*

and should, therefore, be designated as *Ascocotyle pithecophagicola.* In 1918 Tanabe (1922) described a new member of this family, *Stamnosoma armatum* from birds and mammals in Japan, and experimentally infective for man. Recently one of us (Nishigori, 1924) published an extended account of the life cycle of another species of Stamnosoma (*S. formosanum*) from Formosa, morphologically distinct from *S. armatum,* but showing the same capability of infection in birds and mammals, including man. We wish to record also the presence in the Orient of *Pygidiopsis genata* Looss 1907 collected by one of us (Faust) from the dog, at Canton, April 28, 1923. This is a natural infection in a mammal of a fluke of the family Heterophyidae, previously reported only from a bird (*Pelecanus onocrotalus,* Cairo).

In working out the life history of *Stamnosoma formosanum* in 1923 one of us (Nishigori) found two closely related flukes which were recognized as having certain relationships to Stamnosoma, Heterophyes and Metagonimus, but which differed from these genera in several respects, the most important of which was the presence of a single conspicuously large testis instead of the pair of testes commonly described for the group. Life history experiments proved certain fresh-water fishes to be involved in the cycle of infection. During a conference between Doctor Yokogawa and the senior author in September, 1924, Yokogawa asked that the senior author cooperate in the problem, studying phases of the life cycle still incomplete and coordinating this problem with other studies of closely related forms. When it was found that these two species represented a distinct generic type of the family Heterophyidae a decision was reached to propose the name Monorchotrema, for these two species. In a preliminary communication before the Medical Association of Formosa (Nov. 19, 1924) Nishigori designated these species as *Monorchotrema taihokui* and *Monorchotrema taichui,* the specific names referring to the localities in which the flukes were first found. This communication, published in Japanese (Nishigori, 1924a), was followed by a preliminary note published in English (Faust and Nishigori, 1925).

MORPHOLOGY AND LIFE CYCLE OF MONORCHOTREMA TAIHOKUI

Hosts: 1. Mollusc: *Melania reiniana* var. *hidachiens* Pilsbry.
2. Fishes: Cyprinidae, Siluridae, Colitidae and probably members of other families of fresh water fishes.
3. Definitive hosts: Birds (*Nycticorax nysticorax*) and mammals (including dog, cat, rabbit, rat, mouse, guinea-pig and man).
Habitat: Northern and Central Formosa.

The shape of the fluke, when observed as a fixed specimen, preserved in formalin, is flat in both its dorsal and ventral aspects. It is longitudinally elongated, with a constriction near the mid-line separating it into anterior and posterior regions. It is rounded at both the anterior

and posterior ends but is somewhat more truncate posteriorly. In the living worm movement is carried on incessantly, the anterior portion of the body being successively contracted and extended. When it attaches itself to the substratum with its oral sucker the posterior portion of the body is pulled forward. The fluke is very small; when seen creeping over the mucous membrane of the intestine, it looks like a tiny animated mass of yellowish-brown pigment. Its size varies according to age, the number of flukes found in a single host, and the size of the host. But this size difference is not as conspicuous as might be supposed. For example, the mature flukes, which can be obtained experimentally by feeding a mouse with encysted larvae, are generally smaller than those secured by feeding the same cysts to a dog or cat. The reason for the small size of the fluke in the mouse probably lies in the unsuit-

TABLE 1.—*Specimens of Adult Monorchotrema taihokui Fixed in 80 Per Cent Alcohol*

Length (μ)	Width (μ)
449	199
413	215
387	172
466	198
441	206
492	198
413	232
449	232
533	206
501	172
413	172
449	206
466	232
413	232
411	172
Average, 446 μ	Average, 203 μ

ableness of this animal as a host for this species, since the fluke no sooner develops into the adult stage than it is passed in the feces, illustrating the so-called "transit development" in unsuitable hosts.

The living fluke changes its relative dimensions considerably. When elongated it is from 0.52 to 0.62 mm. in length by 0.12 mm. in breadth; when it contracts, the length varies from 0.255 to 0.35 mm., and the breadth from 0.16 to 0.17 mm. By covering it with a coverglass and compressing it slightly so as to enable one to observe its internal structure, the length measurement is increased to 0.674 mm. and the width to 0.355 mm. In specimens artificially fixed, the size of the body differs according to the fixing reagent employed. When it is fixed in hot sublimate, there is always considerable contraction, while in 80% alcohol, the body is sometimes contracted and sometimes extended. In a 4% formalin solution, the worm can usually be fixed in a natural, relaxed condition, measuring 0.394 mm. in length by 0.238 mm. in width (see tables 1 and 2).

The fluke has a rather thick hyaline integument (about 2.8μ in section), homogeneous and transparent. The surface of the body is thickly covered with projecting spines which are recurved and become gradually reduced both in size and in number as they proceed from the anterior to the posterior portion of the body. The spines are conical in shape and are rather thick-set, having a length of 3.3μ and a breadth of 1.6μ. Subintegumentary muscles are found under the hyaline integument, consisting of three layers, transverse, longitudinal and oblique. These muscle layers are not well developed in the posterior portion of the body, but at the anterior end they are quite conspicuous. In the mesenchyme there are large cells, globular or ovoid in shape, which can be easily stained with eosin. There is also a layer of subintegumentary cells under the hyaline membrane, the long axis of which

TABLE 2.—*Specimens of Adult Monochotrema taihokui Fixed in 4 Per Cent Formalin Solution*

Length (μ)	Width (μ)
370	207
387	232
413	241
396	267
396	198
430	249
404	224
404	258
387	258
413	224
387	249
370	249
287	224
396	249
370	241
Average, 394 μ	Average, 238 μ

is at right angles to the surface of the body. These cells are probably secretory in nature.

The oral sucker (Fig. 1, *os*) is circular in shape, very muscular, and situated at the anterior end of the body. It is elevated from the surface of the body and is directed somewhat ventrad. Its length is 54.4μ and its breadth 56μ. A median longitudinal section through the body of the worm shows the well developed radial muscles from the inside of the sucker to the outer wall. It is surrounded by circular muscles. The posterior wall of the sucker is 28μ in section while the anterior wall measures 47μ, which accounts for the fact that the oral sucker is directed posteriad. The ventral sucker (*vs*) is closely connected with the genital sucker like that of *Metagonimus yokogawai*. The shape of the ventral sucker is ovoid or cylindrical. This organ is situated on the right side of the median line of body. Its long axis lies somewhat obliquely, as is shown in figure 5 (*vs*). It measures 55μ in length by 50μ in breadth. The ventral sucker is buried in the parenchyma. Its anterior end opens into the genital sinus. It never

opens to the outer surface of the body, as in the case of other flukes. The anterior end of the ventral sucker is surrounded by spines in the form of chitinous rodlets. The length of these spines is inconsiderable. generally measuring from 1.7μ to 2.5μ. They are therefore easily overlooked, and it is extremely difficult to determine their exact number. It is estimated that there are between thirty-six and forty-two of them.

The mouth lies within the oral sucker, and is somewhat funnel-shaped. Directly inside is the short prepharynx (*pp*) (27μ in length) which in turn is connected with the pharynx (36.2μ in length and 30.6μ in breadth), an elongated object (*p*) which appears reniform in longitudinal section. There is a long esophagus (*e*), having an average length measurement of 76μ. It bifurcates to form the two intestinal ceca (*c*) just in front of the ventral sucker, and extends to the posterior portion of the body, near the anterior border of the testis.

Textfigure 1.—Photomicrograph of mature egg of *Monorchotrema taihokui*. × 920.

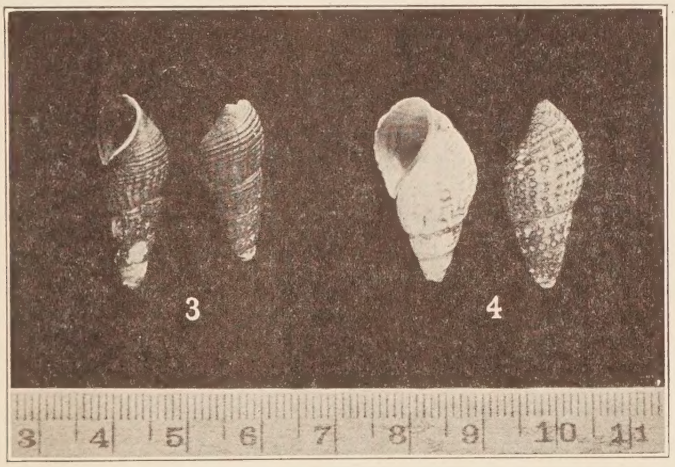
Textfigure 2.—Photomicrograph of mature egg of *Monorchotrema taichui*. × 920.

The excretory bladder (*eb*), in the case of immature individuals is transversely oval, while in mature flukes, on account of the development of the testis, it is compressed along the mid-line and becomes V-shaped. Inside the bladder are many excretory granules, which are highly refractile under the microscope. There are two main ducts (*et*) arising from the bladder, each of which runs forward at each side of the body of the fluke to the anterior end. The anterior portions of the main ducts branch into many capillary tubes, to form a sort of network of capillaries and flame-cells, the exact pattern of which has not been determined. The secretory glands are well developed, especially in the anterior portion of the body. On either side of the esophagus, there are two groups of specially differentiated secretory glands (*cg*), with ducts (*cgd*) opening into the wall of the oral sucker.

REPRODUCTIVE SYSTEM

The genital organs occupy the most prominent position and the most space in the posterior part of the body, the ventral sucker being the only other organ of any importance found in this region.

One of the characteristic features of this fluke is the single large testis (*t*), which is sometimes globular, sometimes compressed ovate in shape. It is situated along the median line in the posterior portion of the body, and is conspicuous for its size. Its length averages 0.184 (0.11 to 0.238) mm.; its diameter amounts to 0.206 (0.13 to 0.272) mm. Its surface is entirely smooth. There are neither lobules nor other irregularities in its outline. The unpaired vas deferens stars from the upper border of the testis, ascends along the left side of the posterior



Textfigure 3.—Oral and aboral views of *Melania reiniana* var. *hidachiens*, intermediate host of *Monorchotrema taihokui*. × 1.

Textfigure 4.—Oral and aboral views of *Melania obliquegranosa*, intermediate host of *M. taichui*. × 1.

margin of the ovary, and opens directly into the posterior part of the seminal vesicle. The seminal vesicle (*sv*), which is in the same plane with the ventral sucker, is irregularly oblong or retort-shaped. It is filled with spermatozoa. The size of the seminal vesicle differs with the amount of contained spermatozoa. In mature flukes the vesicle measures from 0.0925 to 0.204 mm. in length by 0.055 to 0.0675 mm. in lesser diameter. On the posterior border of the left side, is a small "subseminal" vesicle, which is connected with the main vesicle by a thick neck. The right portion of the main vesicle is narrowed, to form the tubular prostate duct. This leads into the ejaculatory duct, which proceeds from the left posterior margin of the ventral sucker to

meet the outer terminus of the uterus. These two tubules abruptly open into the genital sinus. There is neither cirrus nor cirrus sac.

The ovary (*ov*) is situated somewhat to the right of the median line of the body in the posterior part between the testis and the ventral sucker. It is almost spherical in shape, possesses a smooth entire surface, and measures from 0.071 to 0.1 mm. in length by 62 to 87μ in lesser diameter. In the living worm it is characterized by a milky opacity, with granular inclusions. The seminal receptacle (*sr*) is situated on the right side of the body in the same plane with the ovary, or at times somewhat obliquely behind the latter. It is subglobular in shape. Its size varies with the amount of its contents, ranging from 0.459 to 0.595 mm. in length by 0.544 to 0.578 mm. in lesser diameter. It is usually filled with a large amount of spermatozoa. Laurer's canal has its origin in the common duct arising from the ootype and gradually runs dorsad, finally ending blindly just under the body surface. Near its origin there is a slender duct which opens into the right side of the seminal receptacle. The vitelline glands (*vit*) are well developed. They are situated in the posterior portion of the body, beginning at about the same level as the anterior portion of the ovary and the seminal receptacle, usually lying on the dorsal side near the surface of the body. Each lateral half of the vitellaria consists of five or six lobes, each in turn composed of a number of lobules or smaller units, made up of well organized granules. Many small yolk ducts on each side gradually unite to form two ducts, the vitelline ducts, which proceed to the midline along the posterior margin of the ovary and the seminal receptacle, to form the common vitelline duct. This in turn unites with the oviduct on the left side of the ovary. The oviduct arises from the posterior left margin of the ovary, and, joining with Laurer's canal and later with the common vitelline duct, proceeds along the left side of the ovary where it enlarges to form the ootype. The shell gland consists of an irregular mass of single acinous cells, surrounding the ootype, with ducts opening into the latter organ.

The uterus occupies the space between the other organs in the posterior part of the body. It arises from the ootype, proceeds posteriad, then again anteriad, winding back and forth many times in closely folded coils until it finally opens into the genital sinus. In its proximal region it contains immature eggs, which gradually mature as the tube is traced distad. For this reason the eggs in the left side of the uterus (the inner portion) are almost colorless with contents consisting of the blastomeres and the yolk cells, which are granular in appearance. On the other hand the eggs in the portion of the uterus situated on the right side of the body and around the seminal vesicle on the left, are all mature and are consequently yellowish-brown in color and contain viable miracidia. The outer portion of the uterus which

opens into the genital sinus becomes narrowed and its wall becomes slightly thickened. The genital sucker (*gs*) is fused with the ventral sucker, the two forming what is sometimes referred to as the "genital-ventral-sucker-apparatus," in many ways comparable to that in Metagonimus. Its longitudinal axis extends obliquely and parallel to the ventral sucker. At its upper anterior left margin, the genital sucker extends over the opening of the ventral sucker so as to form a big vesicular structure which opens outward through a pore provided with a sphincter. Thus the greater part of this genital sucker lies buried in the mesenchyma. The vesicle measures 61μ in length by about 53μ in width, while the opening has a diameter of 2.8μ. The genital sucker is capable of considerable extensibility, so that the above measurements have been taken from fixed material. At its base the ventral sucker is clearly distinct from the genital sucker, but it is in close contact with the latter, so that in sections the genital sinus has the appearance of a half moon (Fig. 5). Proceeding toward the anterior margin both suckers are closely associated. Along the anterior margin of the ventral sucker, the left wall adheres to the right wall of the genital sucker; and further along a cross section shows only the tissues of the genital sucker without any elements from the ventral sucker. On the anterior face where the genital sucker extends toward the right and covers the anterior margin of the ventral sucker, the former organ attains its greatest breadth. Surmounting the genital sucker is a small genital pore (*gp*). The wall of the genital sucker consists of circular muscle fibers; these are especially well developed at the opening of the organ.

DEVELOPMENT OF MONORCHOTREMA TAIHOKUI

It had been found both by examination of large series of fresh-water snails collected in Taihoku Prefecture and by submitting various snails to experimental infection, that *Melania reiniana* var. *hidachiens* Pilsbry serves as the first intermediate host of this infection (See textfigure 3). The miracidium develops within a small operculate shell, measuring 26 to 29μ in length by 12 to 15μ in transverse diameter. The wall of the shell is very thick, measuring nearly 1.5μ in the posterior half and about 1.3μ in the anterior half (Figs. 2, 3, 12). There is an internal thickening of the shell at the posterior end. There is likewise a conspicuous shoulder thickening, where the operculum joins the shell. It reminds one very much of the shoulder thickening of Clonorchis but differs in being broader and less prominently sculptured than that of the latter species. The operculum is small, having a diameter from 3 to 4μ. Within the shell is the miracidium, which develops at the expense of the yolk cells. Early stages of development of the larva may be seen in eggs found in the proximal coils of the uterus, but as the eggs become crowded into the distal portion they mature rapidly,

so that at the time the eggs reach the genital pore they are usually fully developed.

The mature miracidium (Fig. 12) has a conical anterior half and a hemispherical posterior half. It is covered with a number of long delicate cilia, which are seldom seen in motion before hatching has taken place. The miracidium has a small anterior cone devoid of cilia. Just behind this portion are two lateral ducts (*sgd*), openings of a pair of symmetrical unicellular glands (*sg*). These glands are the secretory glands, producing histolytic ferments which make it possible for the miracidium to penetrate the tissues of the appropriate molluscan host. These glands each have a large hyaline nucleus and a large number of granular cytoplasmic inclusions. In addition to the secretory glands there are several germ cells (*ga*), which fill the posterior part of the body. By focusing on the surface of the mature larva, a number of polygonal cells (Fig. 2) can be seen. These are formed in a definite pattern, are relatively few in number, and constitute the epithelial cells of the miracidium.

The hatching of the miracidium of *M. taihokui* is difficult to observe. This is due in part to the minute size of the larva, which requires the high powers of the microscope for careful observation, and in part to the difficulty in getting normal hatching to occur under the cover-glass. The larva can be "popped" out of its shell by slight pressure of the cover-glass, after which it swims about in water for several minutes before dying. It is realized, however, that this condition is probably not a natural one for the miracidium. When feces containing viable eggs of this species is placed in an aquarium with uninfected *Melania reiniana* var. *hidachiens,* after a period of thirty or forty days, rediae with their cercarial progeny have been found. These cercariae have been proved by experimental methods to encyst on fish and later on to develop into adult *M. taihokui* when this infected fish is consumed uncooked by an experimental mammal. The evidence is therefore not absolutely complete with respect to the intra-molluscan phase of the life cycle, but our data, interpreted in the light of previous knowledge, favor the view that within this period of five or six weeks the miracidium has penetrated the mollusc and has metamorphosed into a sporocyst, which in turn has produced a progeny of rediae such as we have found in our experimental molluscs.

The rediae (Fig. 13), found from dissection of the experimental snails as well as from natural infections, develop to a length of 1.2 mm. and are usually spindle-shaped, although they may be broadly oval on contraction. They contain several mature and maturing cercariae. They are filled with a golden brown pigment, which is particularly conspicuous in their rhabdocoel gut, but they are much lighter in color than the deep brownish-yellow of the liver cells of the snails, in the inter-

hepatic lymph spaces of which they develop. Aside from the well formed pharynx (*p*) and the gut (*c*) there are no special organs by which these rediae can be differentiated from those of other species of this family.

The cercaria of *M. taihokui* is a lophocercous larva (Fig. 14), with a long muscular tail having a fluted, keeled margin. It also possesses a pair of pigmented eye-spots (*es*), situated on the dorsal side some little distance behind the anterior sucker. When the body becomes elongated it looks very much like an elephant's proboscis and measures 0.278 mm. in length by 0.058 mm. in greatest width. When it becomes constricted it looks more like a tadpole and then measures 0.129 mm. in length by 0.074 in width. When fixed in 4% formalin solution it becomes almost cylindrical in shape. The size of ten mature individual fixed by this method is shown in Table 3.

TABLE 3.—*Measurements of Ten Mature Cercariae of Monorchotrema taihokui Fixed in 4 Per Cent Formalin Solution and Examined Under a Cover-Glass*

Body		Tail	
Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)
189	77	327	32
215	86	447	29
206	96	430	25
189	77	413	27
198	69	473	30
206	77	456	29
215	74	464	29
198	74	496	27
215	86	464	32
189	69	430	27
Average, 202 μ	Average, 79 μ	Average, 440 μ	Average, 29 μ

The body is colored with a yellowish-brown pigment, and is entirely covered with fine reversed spines; these are especially thickset at the anteriomost part of the body. The oral sucker (Fig. 15, *os*) is at the anterior end of the body and is directed somewhat ventrad. It measures 39μ in length and 38μ in width. Its depth is practically that of its transverse diameter. The ventral sucker (*vs*) is not so clearly seen as the ventral acetabulum of most distome cercariae, but if it is carefully searched for it may be found some little distance behind the center of the body (Fig. 14, *vs*). It measures up to 63μ in diameter. The digestive system can be recognized for only a short distance behind the oral sucker, where the prepharynx and minute pharynx are to be seen. The remainder of the intestinal canal is obscured by glandular tissues. On both sides of the body there are comparatively large cell groups which surround the ventral sucker and the excretory bladder. These are the cephalic glands (*cg*), seven in number on each side of the body. The cephalic gland ducts (*cgd*), which arise from each gland

cell, proceed toward the anterior extremity of the body where they open separately through the dorsal wall of the oral sucker. One of us (Nishigori), in collaboration with Professor Yokogawa, has studied this structure in living cercariae and finds that the details correspond to those worked out by the senior author on preserved material of the cercariae of *M. taichui,* namely, that the oral opening (*os*) is provided with a piercing apparatus, in the dorsal wall of which are four needle spines, while immediately dorsal to the orifice are two alternating rows of five (lower) and six (upper) thorn-like spines. Elements of the genital cells (*ga*) are found in stained specimens in two positions: (1) just behind the middle of the body, and (2) just behind the plane of the eye-spots.

The tail is fitted into a hollow groove at the posterior end of the body. It is grayish in color, measures about 0.44 mm. in length by 0.029 mm. in width, and becomes gradually narrowed as its distal end is approached. As previously stated, there is a wing-like fluted membrane on either side of the tail, which aids in locomotion. Movement consists in a forward motion brought about by the constant jerky beating of the tail.

The distribution of cercariae of *Monorchotrema taihokui* is very extensive, as has been proved by examination of *Melania reiniana* var. *hidachiens* gathered from the northern and central regions of Formosa. The amount of infection in these molluscs differs according to the season and to the habitat where they are gathered. We have found that out of 1,048 snails which were collected in the suburbs of Taihoku City twenty-one snails were infected (about 2%). If large numbers of mature cercariae of *M. taihokui,* which have escaped from the snail *Melania reiniana,* are put into a receptacle in which gold-fishes are kept, after swimming about for a while these cercariae crowd around the fishes, and migrate to the position where they are found encysted in nature, namely, attached to the cartilage of the caudal, ventral, thoracic and dorsal fins. It is unusual to find them attached to the outer portion of the fins. In addition, large numbers of cysts are occasionally found attached to the head, the palate and the gill arches. Only seldom does one find the cysts on the scales, or in the superficial flesh and then only in small numbers. Inspection of the cartilage of the fin, soon after the larva has penetrated, reveals the presence of the larva which has already lost its tail. This was accomplished by a violent agitation of the organ, together with extension and contraction of its body at the time it invaded the cartilage tissue. Invasion of the tissue is accomplished by a secretion of histolytic ferments from the cephalic glands, through the duct openings around the oral sucker (Fig. 15, *cgo*). After a period of coiling and twisting, now and then involving a rotary motion, the cercaria progresses into the tissues, at the same time, gradually

secreting its cyst capsule. Already in twenty-four hours after it has attacked the fish the cyst capsule is visible, although still somewhat obscure. In three or four days, it is complete. In this way, in the course of time, changes of the structure of the larva and of the diameter of its cyst capsule are found to occur. Measurements illustrating the size of the cyst at various times after invasion of the fish are presented in Table 4. They were made from living specimens under slight pressure of a cover-glass. The average figure is shown for fifteen measurements each.

Four days after the larvae had penetrated the host tissues, the cyst wall had been completely formed. In structure it is a transparent, hyaline, homogeneous capsule, perfectly formed, measuring 5μ in thickness without further increase. On the other hand, the length and width of the cyst increase in course of time; in twenty-four hours after

TABLE 4.—*The Size of Cysts During Successive Periods After the Larva of M. taihokui Has Penetrated the Fish Host*

Day	Length (μ)	Breadth (μ)	Thickness of the Cyst Wall (μ)
20th hour	87.5	78.0	3.76
4th day	108.6	86.4	3.80
7th day	129.7	114.7	5.00
10th day	132.9	116.7	5.00
13th day	136.3	115.0	5.00
16th day	136.3	116.4	5.00
19th day	147.9	122.3	5.00
20th day	147.9	132.1	5.00
24th day	150.0	137.5	5.00

infection it grows to a length of 88μ and a breadth of 78μ; while in ten days it has attained a length of 133μ and a breadth of 117μ. Fully mature cysts reach a length of 148μ and a breadth of 132μ. This increase may proceed until they have attained a size two and a half to three times that at the time of invasion (Figs. 16-18). The adolescariae enclosed within their cyst capsules, undergo structural changes gradually leading to maturity. Twenty-four to twenty-eight hours after penetration and encystment the eye spots are still plainly visible, but the ventral sucker and intestinal canal are somewhat obscure. The excretory bladder is oval in form; the cephalic salivary glands on each side of the body are still present. In other words, at this time these organs have changed little from those of the mature cercaria. On the third or the fourth day, however, both the length and the width of the larva have considerably increased. The cephalic glands have become inconspicuous, but the excretory bladder is still visible. Between the fifth and the seventh days, the pigment granules of the eye spots begin to disappear, while the pigment granules in the body decrease in amount. In some individuals that have attained precocious maturity, highly

refractive hyaline granules may be observed in the intestinal ceca. Between the eighth and the tenth days, the body has nearly attained final form. About the tenth or the eleventh day, the excretory bladder becomes enlarged; the ventral sucker (*vs*) has already assumed an oblique position on the right side of a body. Near the fifteenth day, the encysted adolescariae become fully mature. The worms may be seen within the cyst capsule, rotating nervously, the integumentary surface thickly covered with spines, the excretory bladder full of highly refractile darkly colored granules. The genital and ventral suckers are plainly discernible, while the coronet of small spines around the genital sucker is distinctly recognizable. At this time even the primordium of the testis becomes differentiated. The process of development briefly outlined above for the encysted adolescaria is affected by the temperature of the water, as well as by the climate and the season. The experimental data herein recorded have been obtained during the month of May, when the laboratory temperature in Formosa ranges from 27 to 30 C.

The second intermediate hosts are the fresh-water fish in Formosa and most of those collected to date have proved to harbor the cysts of this fluke. They include *Carassius auratus* (Linnaeus), *Clarias fuscus* (Lacepede), *Channa formosana* (Jordan and Evermann), *Pseudorasbora parva* (Schlegel), *Phodens ocellatus* (Kner), *Gambusia affinis* (Baird and Girard), *Polyacanthus operculatus* (Linnaeus), *Ctenophalus tadianus* (Jordan and Evermann), *Misgurnus anguillicaudatus* (Cantor), *Parasilurus asotus* (L), *Zacco platypus* (T. and S.), *Cypinus carpio* (L.). Besides these it seems probable that still others serve as second intermediate hosts, because the cysts of this fluke has proved to be extensively distributed among the three families, Cyprinidae, Siluridae and Colitidae.

When the encysted adolescariae of this fluke have been taken into their final host, they are first subjected to the medium of the gastric juice, which, however, fails to free the young flukes from their cysts. After entering the intestine, the cyst capsules are rupture and the flukes set free, creeping over the mucous membrane of the intestine or invading deeply between the villi, where they gradually develop to adulthood. About the third or fourth hour after an experimental feeding of the cysts of this fluke the size and structure of the worm does not differ materially from that immediately following excystment, although the intestinal and excretory concretions have decreased or are decreasing in number and size. Aside from the single mass of testis cells and the obliquely directed ventral sucker little detail of structure is visible. After twelve hours, however, the mass of the testis increases to such a size that the excretory bladder becomes disposed dorsal to it and

narrowed in outline, while the mass of the ovary becomes somewhat conspicuous. In seventy-two hours after an experimental feeding, the testis and ovary are much enlarged, and the excretory bladder becomes pressed into a V-shaped structure. In addition, the seminal receptacle now assumes a circular outline. In the uterus two or three immature eggs are seen; the developed vitellaria are also recognizable. On the sixth day after an experimental feeding, all of the organs in the fluke's body have practically developed to maturity. Slightly immature flukes are found containing more than sixty eggs in the uterus, in the outer end of which some yellowish-colored mature eggs are found. The uterus rapidly becomes elongated with a corresponding increase in the number of its coils. Ten to thirteen days after an experimental feeding, all the flukes have become mature; each organ has become perfectly developed; the single large testis is seen in the posterior part of the body, translucent white in color. The excretory bladder shows the characteristic V-shaped cleft. On the right side of the body there is a big seminal receptacle; the seminal vesicle is elongate cylindrical in shape; both the seminal receptacle and the seminal vesicle are full of spermatozoa moving about in whirls. In the vicinity of the genital sucker, three or four mature eggs and many spermatozoa may be seen. The uterus at this time contains from two hundred to three hundred eggs.

MORPHOLOGY AND LIFE CYCLE OF MONORCHOTREMA TAICHUI

Hosts: 1. Mollusc: *Melania obliquegranosa* (Smith).

2. Fish: *Cyprinus carpio, Carassius auratus, Zacco platypus, Pseudorasbora parva, Phodeus ocellatus, Gambusia affinis, Ctenopharyngodon idellus.*

3. Definitive: Birds (?), mammals, including man (experimental infection).

Habitat: Northern and Central Formosa.

The fluke, *Monorchotrema taichui* bears a very close resemblance to *M. taihokui,* with respect to size, structural characteristics, movement, life cycle, free-swimming miracidial and cercarial stages, and the manner of its development in the definitive host. We shall, therefore, include here only those particulars in which it differs from *M. taihokui.*

The body of the fluke, when naturally relaxed, is oval in shape, with flattened dorsal and ventral sides. There is at times a slight constriction between the anterior and posterior portions. The fluke is small, differing little from *Monorchotrema taihokui.* The living worm is very changeable, varying from 1.054 to 1.18 mm. in length by 0.17 mm. in width when extended, and 0.425 mm. in length by 0.255 mm. in width, when contracted. Upon mounting it on a slide with a cover-glass and compressing it so as to enable one to observe its internal structure, we find that its length averages 0.723 mm. and its width 0.306 mm. When it is fixed, the size of its body differs according to the kind of fixing

reagent. If the worms are fixed in 4% formalin, the body will assume a natural shape, measuring on an average 0.352 mm. in length by 0.234 mm. in width (Tables 5, 6).

The worm has a hyaline integument from which there have grown out minute reversed spines covering the whole surface of the body. Underneath this layer there are subintegumentary muscles, identical with those of *Monorchotrema taihokui*. The oral sucker is circular

TABLE 5.—*Measurements of Adult Specimens of Monorchotrema taichui Fixed in 80 Per Cent Alcohol*

Length (μ)	Width (μ)
447	231
387	215
516	231
396	230
413	231
430	231
420	267
473	275
447	275
413	231
516	230
387	231
413	215
473	215
447	231
Average, 443 μ	Average, 236 μ

TABLE 6.—*Measurements of Adult Specimens of Monorchotrema taichui Fixed in 4 Per Cent Formalin*

Length (μ)	Width (μ)
340	272
357	255
340	255
391	255
374	255
357	238
340	272
374	272
340	204
340	187
374	255
306	187
255	187
240	221
Average, 352 μ	Average, 234 μ

in shape, very muscular, and directed somewhat ventrad. In living specimens it measures 59μ in length by 65μ in width; in fixed specimens it measures 52μ in length by 60μ in width. In median longitudinal sections the thickness of the oral sucker is about the same on its dorsal as on its ventral aspect (ca. 14μ), the dorsal wall considerably exceeds the ventral wall in depth, which fact accounts for its inclined position. The opening of the oral sucker is funnel-shaped.

The ventral sucker is peculiar, as it is in *M. taihokui* and *Metagonimus yokogawai*. It is closely associated with the genital sucker. Its

shape is oval or circular. It is situated to the right of the median line and measures 69μ in length by 5μ in breadth. The whole of the ventral sucker is buried deeply in the mesenchyma. It lies with its long axis inclined obliquely (Fig. 9, *vs*). In transverse section it is almost symmetrically round; the left edge is near the median line; the posterior portion is enlarged, and is inclined to the right. The orifice is club-shaped, but is enlarged at its base. Its wall is thick, consisting chiefly of circular and radial muscles. There are conspicuous keel-shaped spines surrounding the ventral sucker where the genital sucker is fused with it. The spines on both margins of the semicircular crown are shorter than they are in the center. The shortest is 0.7μ in length and the longest is 1.8μ in length. They number about 11, but are so short on both edges that a count is subject to error (Fig. 9). The length of each spine as measured in an average specimen, from above downward, is as follows:

No. I 0.7 μ.	No. V 1.2 μ.	No. IX 1.6 μ.
No. II 0.9 μ.	No. VI 1.3 μ.	No. X 1.4 μ.
No. III 1.0 μ.	No. VII 1.7 μ.	No. XI 1.0 μ.
No. IV 1.1 μ.	No. VIII 1.8 μ.	

Monorchotrema taichui is like *M. taihokui* in that these spines are on the ventral sucker and not on the genital sucker.

The structure, formation and situation of the oral cavity, the prepharynx (length, 306μ), the pharynx (length, 42.9μ, width 28.6μ) and the esophagus, are all similar to those in *Monorchotrema taihokui.* The esophagus bifurcates in front of the ventral sucker into two intestinal ceca which run into the posterior portion of the body. The course of the main excretory vessels, the formation of the excretory bladder and the capillaries and flame-cells are very similar to those of *M. taihokui.* The glands are also indistinguishable from those of *M. taihokui.*

As in *Monorchotrema taihokui,* the testis is single (*t*). Its shape is round; it is situated on the median line in the posterior portion of the body; it is very large, measuring 0.169 mm. in length by 0.156 mm. in breadth and 0.13 mm. in thickness. Although it is difficult to determine the course of the sperm duct it appears to commence as a single duct (*vas efferens, ve*), starting from the upper part of the testis, then passing along the left side of the ovary and, finally opening into the posterior end of the seminal vesicle. The latter (*sv*) is situated at the lower border of the ventral sucker and is directed toward the left side of the body. At its center it becomes constricted and divided into right (anterior) and left (posterior) elements. The right elements appears to be always larger than the left. The right (anterior) side becomes narrowed to form the tubular prostate duct. It is continued forward and ventrad as the ejaculatory duct, which opens into the genital sinus. There is neither cirrus organ nor cirrus sac.

The ovary (*ov*) is situated on the right of the median line of the body, behind the ventral sucker. It measures 69μ in length by 77μ in width. The seminal receptacle (*sr*) is situated on the right side of the body, and is in the same plane with the ovary or somewhat posterior to it. Its form is circular. In living specimens its contents of spermatozoa are constantly in motion. It measures 57μ in length by 61μ in width. The structure and course of Laurer's canal are about the same as in *Monorchotrema taihokui.* The vitelline glands (*vit*) are well developed. They are situated in the posterior part of the body, extending backward from the upper border of the ovary and the seminal receptacle, and are especially conspicuous along the margin of the body. Each side consists of four or five lobes of glands. Many small yolk ducts converge to form two ducts, one from the right and the other from the left side of the body, joining together to form the common yolk duct, which enters the ootype near the opening of the oviduct. The oviduct starts from the median left margin of the ovary, and after receiving Laurer's canal, combines with the common yolk duct to enter the ootype. The shell gland is an irregular group of single cells situated around the ootype, with ducts opening into the latter organ. The uterus issues from the ootype along the upper margin of the testis and to the left of the ovary; its course follows the same general plan as that of *Monorchotrema taihokui.*

The genital sucker covers the anterior half of the ventral sucker and forms a part of the *"ventral-genital-sucker-apparatus."* Some mature flukes contain several eggs in the genital sinus. The wall of the uterus which opens into the genital sinus is slightly thickened. The genital pore which opens outward measures in fixed specimens about 2.8μ in diameter. In this region, the body spines become shortened and fewer in number. The wall of the genital sinus has well-developed circular and longitudinal muscles. In the region where the genital and ventral suckers come in contact, the circular muscles are best developed, forming a belt-like sphincter.

DEVELOPMENT OF MONORCHOTREMA TAICHUI

The first intermediate host of *Monorchotrema taichui* is a Melania. As far as observation has been made the parthenitic generations develop only in *Melania obliquegranosa* (Smith) (Textfig. 4). The miracidium which hatches from the egg (Textfig. 2) penetrates the tissues of the snail to which it is adapted, as in the case of the miracidial larva of *M. taihokui,* and metamorphoses into a sporocyst. Rediae are produced parthenogenetically within these sporocysts, and after a period of five to six weeks mature cercariae develop within the rediae. These cercariae erupt from the snail tissues and are found freely swimming about in the

water. The mature cercaria (Fig. 20) is a lophocercous larva with an oval-elongate body and a tail which whips about violently. This cercaria closely resembles that of *Monorchotrema taihokui* but its body is only about two thirds as large. On elongation the living cercaria becomes cylindrical (0.2 mm. in length by 0.05 mm. in width), and the anterior end gropes about like an elephant's proboscis. On contraction, it takes the form of a tadpole (0.1 mm. in length and 0.09 mm. in width). On becoming quiescent or when mixed in 4% formalin solution, the body becomes broadly cylindrical. Such specimens have the following measurements (Table 7).

TABLE 7.—*Measurements of Ten Mature Cercariae of Monorchotrema taichui Fixed in 4 Per Cent Formalin Solution, Examined Under a Cover-Glass*

Body		Tail	
Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)
139	50	360	19
133	78	340	21
125	55	390	21
139	55	370	19
172	58	340	19
133	58	410	67
144	78	390	21
139	66	390	19
133	72	370	19
146	66	390	21
Average, 140 μ	Average, 64 μ	Average, 366 μ	Average, 25 μ

The body is colored with yellowish-brown pigment, which is more conspicuous than that in the cercaria of *M. taihokui*. The surface of the body is covered with tiny reversed spines. There is a pair of pigmented eye spots (*es*), situated to the right and to the left of the pharynx. The oral sucker (*os*) is found at the anterior end of the body and is inclined somewhat ventrad. It measures 0.14 mm. in length by 0.064 mm. in width. The ventral sucker (*vs*) is indistinctly seen as is the condition in the cercaria of *Monorchotrema taihokui;* the digestive system as far as can be found, consists of the prepharynx and pharynx. This cercaria has cephalic secretory glands, arranged in a similar manner to those of the cercaria of *M. taihokui* (Fig. 20, *cg*). They fill the posterior half of the body, lateral to the genital mass and the bladder. There are seven pairs of these glands, with contents oxyphilic in reaction. Conspicuous ducts from these cells extend on each side of the body toward the anterior end, where they pass through the oral sucker and open outward above the orifice of the digestive tract (Fig. 22, *cgd, cgo*).

A careful study of preserved stained specimens, examined with an oil immersion objective, reveals an interesting relationship of these several organs at the anterior end of the body. Figures 21 and 22 show respectively a head-end and a profile view looking into the orifice. The

anteriormost portion of the body bends ventral. On the dorsal face this consists of a graceful curve; on the ventral, it constitutes a definite angular bend. A lateral view (Fig. 22) indicates that the cephalic secretory gland ducts (*cgd*) curve downward parallel to the dorsal curve of the body, but just before opening to the surface bend abruptly dorsad, and open through constricted reinforced tips (i. e., reinforced capillary tubes). The openings are separated a considerable distance from the oral opening (Figs. 21, 22, *oo*), which is itself provided with a reinforced piercing apparatus, consisting of two fused lips, a short thick dorsal one and a flat ventral one. Inserted into the dorsal lip are four acicular spines, while immediately dorsal to the orifice are two alternating rows of five (lower) and six (upper) thorn-like spines. This whole system of organs evidently constitutes the invasion apparatus of the cercaria, the modification of the orifice of the digestive tract serving as an abrading and attachment organ to supplement the histolytic ferments secreted by the cephalic glands. There is further evidence that the modified opening of the digestive system as well as the cephalic gland secretions is utilized for penetration and attachment at this period of transfer to the piscine host, in view of the fact that the whole apparatus breaks down and disappears soon after the larva encysts. This supplementary apparatus, consisting of a temporary modification of the orifice of the digestive tract, is apparently unique for this group of cercariae, and to our knowledge, has not been previously recorded in the literature.

The tail (*ca*) of the cercaria of *M. taichui* is fitted into the hollow groove at the posterior end of the body. It is grayish in color and is uncommonly large for the body of the cercaria, measuring on the average 366μ in length by 25μ in width at its base and becoming gradually narrowed down toward its distal end. There is a wing-like fluted membrane on either side of the tail along its entire length, similar to the marginal keel of the cercaria of *M. taihokui*. The cercarial stage of *Monorchotrema taichui* has a wide distribution, as determined by the relatively heavy infection of *Melania obliquegranosa* collected from the streams in the north and in the central parts of Formosa. Out of 792 snails which were gathered from the mountain streams at Gaishatai, eighty-one (about 10%) were found infected with this fluke.

If the cercariae of *Monorchotrema taichui,* which mature and are set free from the snail, *Melania obliquegranosa,* are put into a receptacle in which gold-fishes are kept, these cercariae soon become crowded together around the fishes and finally invade their tissues. The method of invasion and the position where they come to reside in this species of host are identical with the similar conditions in *Monorchotrema taihokui,* i. e., they become encysted on the cartilage of the fins and on the cartilage tissue of the palate and the gill filaments. Inasmuch as the

encystment of these cercariae and their transformation is similar to these processes in *Monorchotrema taihokui* they will be described very briefly here. The changes in the size of the cysts, depending on the number of days of development in the fish, are shown in Table 8.

The transformation of the cercaria in the cyst resembles that of *Monorchotrema taihokui,* but the disappearance of the pigmentation of the eye-spots seems somewhat more delayed than in *Monorchotrema taihokui,* since twelve days after encystment the pigmentation is still present. The first appearance of the germinal mass is altogether similar to that of *Monorchotrema taihokui.* The development of the spines, which are situated on the ventral sucker, can be traced from the thirteenth to the fifteenth day after the cercaria has invaded the fish.

TABLE 8.—*The Size of the Cysts of Monorchotrema taichui for Successive Periods Following Penetration of the Cercariae and Their Encystment in the Fish*

Day	Length (μ)	Breadth (μ)
24 hours	85.0	64.5
2nd day	86.1	69.2
4th day	118.8	88.4
6th day	118.0	95.8
8th day	137.1	109.6
10th day	143.5	110.1
12th day	148.0	121.2
14th day	148.1	121.2
15th day	158.4	135.6
17th day	156.1	133.2
19th day	150.9	136.5

With regard to the second intermediate hosts of this fluke, seven species of fish, collected from the area of Taichu, have been found naturally infected. They are *Cyprinus carpio* (Linn.), *Carassius auratus* (Linn.), *Zacco platypus* (T. & S.), *Pseudorasbora parva* (Schlegel), *Phodeus ocellatus* (Kner), *Gambusia affinis* (Baird and Girard), *Ctenopharyngodon idellus* (C. & V.). Besides these, the gold-fish, a variety of *Carassius auratus,* is capable of serving as the second intermediate host of this fluke.

The development of the adolescaria of *M. taichui* in the body of its final host is similar to that of *Monorchotrema taihokui.* During the first four hours after an experimental feeding, the fluke does not differ in size and structure from the larva when first freed from the cyst. During this period the oral sucker, the pharynx, the ventral sucker, the testis and the ovary are already distinctly recognizable and further development consists in the enlargement and maturing of these organs. After forty-eight hours the ovary is plainly visible and two or three coils of the uterus can be recognized. In precocious individuals the seminal vesicle is differentiated at this period. On the fourth day after an experimental feeding, the testis is enlarged, measuring 120μ in diameter. The seminal vesicle is constricted in the middle. The ovary

and the seminal receptacle are greatly enlarged, two or three immature eggs are seen in the uterus, and the vitellaria are well developed along both side of the body. On the fifth or the sixth day after the experimental feeding, all of the organs in the fluke's body have developed nearly to maturity. Both the seminal vesicle and the seminal receptacle are filled with an abundance of spermatozoa. Fifty to seventy eggs can be counted in the uterus, eggs of a yellowish-brown color. On the seventh or the ninth day the flukes have become fully mature. There are many eggs in the uterus. Those of a yellowish-brown color are crowded into the outer half of the uterus so that the right side of the posterior part of the body has a yellowish-brown tinge. There are two or three eggs in the genital sinus.

DEFINITIVE HOSTS AND INFECTION OF THE HUMAN BODY

Four specimens of *Nycticorax nycticorax* were caught in Taihoku Prefecture, of Formosa, and were examined for intestinal parasites. The search revealed many *Monorchotrema taihokui* in the middle and the lower parts of the small intestine of one of these birds. On another day a natural infection of these flukes was also found in the small intestine of two dogs and a cat which had been used for other purposes in the laboratory. One dog was infected with more than twenty *Monorchotrema taihokui* and the other with several specimens of *M. taichui,* while the cat was also infected with several specimens of *M. taichui.* From these natural and experimental infections, it is evident that these two flukes possess similar host potentialities. It seems likely that both of these species may utilize birds, dogs and cats as their normal definitive hosts. Experimental evidence shows that rabbits, rats, mice and guinea-pigs can also serve as final hosts of these flukes.

Since no natural infection of these flukes in the human body was on record, on Oct. 3, 1924, one of us (Nishigori) swallowed the cartilage tissue of four gold-fish which contained a great many of the encysted larvae. Examination of the feces on the seventh and on the ninth day after experimental ingestion of the cysts was negative. On the fifteenth day, however, out of five grams of feces, three eggs of *Monorchotrema taihokui* and four of *M. taichui* were found. There is experimental proof, therefore, that these two species of flukes are capable of infecting the human body.

As above mentioned, *Monorchotrema taihokui* and *M. taichui* both choose the small intestine of various animals, including man, for their definitive habitat, particularly in the middle and lower parts. Moreover, in order to confirm their exact position in the intestinal canal, examination was made of the numbers of these parasites found at various levels of the small intestine of experimental animals (See Tables 9, 10, 11, 12).

TABLE 9.—*The Number of Individuals of Monorchotrema taihokui and the Position of These Flukes in the Small Intestine of Experimental Dogs and Cats*

Animal	Day After Experimental Feeding	The Distance from the Pyloric Region and the Number of Parasites Found						
		1–25 cm.	26–50 cm.	51–75 cm.	75–100 cm.	101–125 cm.	126–150 cm.	151–175 cm.
Dog 3.....	7th day	(24 cm.)	(48 cm.)	(72 cm.)	(96 cm.)	(120 cm.)	.....	.....
		0	0	8	13	5	.....	.....
Dog 2.....	11th day	(15 cm.)	(30 cm.)	(60 cm.)	(90 cm.)	(105 cm.)	.....	.....
		0	0	3	15	6	.....	.....
		.....	(45 cm.)	(75 cm.	.....	.....	.....	.....
		.....	0	16	.....	.....	.....	.....
Dog 4.....	30th day	(10 cm.)	(30 cm.)	(60 cm.)	(80 cm.)	(110 cm.)	(130 cm.)	(152 cm.)
		1	57	133	422	60	6	0
		(20 cm.)	(40 cm.)	(70 cm.)	(90 cm.)	(120 cm.)	(140 cm.)	.....
		17	123	102	279	6	1	.....
		.....	(50 cm.)	.....	(100 cm.)	.....	.....	.....
		.....	117	.....	96	.....	.....	.....
Dog 5.....	47th day	(10 cm.)	(30 cm.)	(60 cm.)	(80 cm.)	(110 cm.)	(130 cm.)	(156 cm.)
		0	21	61	52	124	72	0
		(20 cm.)	(40 cm.)	(70 cm.)	(90 cm.)	(120 cm.)	(140 cm.)	.....
		6	62	54	82	134	12	.....
		.....	(50 cm.)	.....	(100 cm.)	.....	(150 cm.)	.....
		.....	52	.....	142	.....	0	.....
Cat 3.....	8th day	(5 cm.)	(30 cm.)	(55 cm.)	.....	.....	.....	.....
		0	0	0	.....	.....	.....	.....
		(10 cm.)	(35 cm.)	(60 cm.)	.....	.....	.....	.....
		0	0	1	.....	.....	.....	.....
		(15 cm.)	(40 cm.)	(65 cm.)	.....	.....	.....	.....
		0	0	0	.....	.....	.....	.....
		(20 cm.)	(45 cm.)	(70 cm.)	.....	.....	.....	.....
		0	0	0	.....	.....	.....	.....
		(25 cm.)	(50 cm.)	.....	.....	.....	.....	.....
		0	5	.....	.....	.....	.....	.....
Cat 2.....	20th day	(5 cm.)	(30 cm.)	(60 cm.)	(80 cm.)	.....	.....	.....
		0	0	70	59	.....	.....	.....
		(10 cm.)	(40 cm.)	(70 cm.)	(89 cm.)	.....	.....	.....
		0	2	102	13	.....	.....	.....
		(20 cm.)	(50 cm.)	.....	.....	.....	.....	.....
		0	4	.....	.....	.....	.....	.....

TABLE 10.—*The Number of Individuals of Monorchotrema taihokui and the Position of These Flukes in the Small Intestine of Experimental Mice*

Animal	Day After Experimental Feeding	The Distance from the Pyloric Region and the Number of Parasites Found				
		1–5 cm.	6–10 cm.	11–15 cm.	16–20 cm.	21–25 cm.
Mouse 10.....	24th hour	(3 cm.)	(6 cm.)	(12 cm.)	(18 cm.)	(21 cm.)
		0	3	3	0	0
		.....	(9 cm.)	(15 cm.)	.....	(24 cm.)
		.....	7	0	.....	0
Mouse 4.....	3rd day	(5 cm.)	(10 cm.)	(15 cm.)	(19 cm.)	.....
		8	27	17	11	.....
Mouse 6.....	6th day	(3 cm.)	(6 cm.)	(12 cm.)	(18 cm.)	(21 cm.)
		0	0	0	0	0
		.....	(9 cm.)	(15 cm.)	.....	(24 cm.)
		.....	0	0	.....	33
Mouse 8.....	6th day	(3 cm.)	(6 cm.)	(12 cm.)	(18 cm.)	(21 cm.)
		0	0	1	8	4
		.....	(9 cm.)	(15 cm.)	.....	(24 cm.)
		.....	0	5	.....	25
Mouse 7.....	8th day	(3 cm.)	(6 cm.)	(12 cm.)	(18 cm.)	(21 cm.)
		0	5	34	6	9
		.....	(9 cm.)	(15 cm.)	.....	(24 cm.)
		.....	16	10	.....	19
Mouse 13.....	13th day	(3 cm.)	(6 cm.)	(12 cm.)	(18 cm.)	(21 cm.)
		0	0	0	0	0
		.....	(9 cm.)	(15 cm.)	.....	(24 cm.)
		.....	0	0	.....	0
Mouse 12.....	15th day	(3 cm.)	(6 cm.)	(12 cm.)	(18 cm.)	(21 cm.)
		0	0	0	0	0
		.....	(9 cm.)	(15 cm.)	.....	(24 cm.)
		.....	0	0	.....	0

TABLE 11.—*The Number of Individuals of Monorchotrema taichui and the Position of These Flukes in the Small Intestine of Experimental Cats*

Animal	Day After Experimental Feeding	The Distance from the Pyloric Region and the Number of the Parasites Found									
		1–10 cm.	11–20 cm.	21–30 cm.	31–40 cm.	41–50 cm.	51–60 cm.	61–70 cm.	71–80 cm.	81–90 cm.	91–100 cm.
Cat 5	7th day	8	14	59	62	143	83	36	31	6	(96 cm.) 0
Cat 2	26th day	0	5	7	22	21	56	30	0	0	(94 cm.) 0
Cat 4	45th day	6	1	7	8	21	9	1	(79 cm.) 0		
Cat 1	89th day		(15 cm.) 0	(30 cm.) 0		(45 cm.) 27	(60 cm.) 44		(75 cm.) 78	(90 cm.) 16	

The flukes invade the upper part of the small intestine during the first period of their infection but as they mature, they become more and more common in the middle and lower parts. *Monorchotrema taihokui* appears to invade the lower part of the jejunum more frequently than the upper part, while *Monorchotrema taichui* invades principally the middle part of the jejunum. It is difficult to find the flukes in the lower levels of the small intestine of the dog and the cat, but when the mouse was used as experimental host both species were found to reside more commonly in the lower levels and less commonly in the middle part. This is a reasonable expectation, since both species during their transitional development from time to time becomes gradually excreted into the lumen of the intestinal canal along with mucus and other exudates and then secure a reattachment further down.

TABLE 12.—*The Number of Individuals of Monorchotrema taichui and the Position of These Flukes in the Small Intestine of Experimental Mice*

Animal	Day After Experimental Feeding	The Distance from the Pyloric Region and the Number of the Parasites Found			
		1–6 cm.	6–12 cm.	12–18 cm.	More Than 18 cm.
Mouse 12	24th hour	.....	.....	.....	(20.5 cm.)
		8	19	6	8
Mouse 10	4th day	5	8	0	0
Mouse 3	5th day	0	4	0	0
Mouse 11	3rd day	1	4	3	6
Mouse 13	5th day	1	3	2	13
Mouse 14	7th day	0	0	0	2
Mouse 15	9th day	0	0	2	0

Note.—There is considerable evidence from Table 12 that the mouse is only a semi-suitable host for this fluke.

The flukes invade deeply the mucous membrane and become attached. At times there are seen in the mucous membrane many eosinophiles and leukocytes, but no marked pathological change is recognizable. Furthermore, the intestinal epithelium is at times slightly atrophied, and wide stretches of solitary intestinal glands are occasionally seen. Some flukes, which have invaded the mucous membrane, again come to lie with their heads attached to the surface of the mucous membrane. The position of these worms in the hosts tissues is, therefore, similar to that of *Metagonimus yokogawai.* Thus the pathological change due to the presence of these worms is so slight, that it seems evident its clinical importance to the host is almost negligible. It is important, however, to differentiate the eggs of these two flukes from those of *Clonorchis sinensis* and of *Metagonimus yokogawai,* which have been shown to be of clinical significance.

THE EGGS AND THEIR DIFFERENTIATION

The mature eggs of *Monorchotrema taihokui* which appear in the feces, are light yellowish or light yellowish-brown in color, almost ovoid in shape, with a narrowed front part, which increases in diameter toward

their posterior portion (Figs. 2, 3; Textfig. 1). The egg shell is comparatively thick (1.3 to 1.5μ), increasing in thickness from the front backward. Some eggs possess internal thickenings on the posterior end. There is an operculum like a watch-glass at the anterior end. Where the operculum joins the shell there is a distinct thickened shoulder, as is seen in an egg of *Clonorchis sinensis,* although in the egg of *M. taihokui* the shoulder is broader and clings more closely to the shell. The average size of the egg is 27μ (24.5 to 29μ) in length, by 13.5μ (12.2 to 15.3μ) in diameter. Within the egg-shell there is a mature miracidium, which has been described.

The mature eggs of *Monorchotrema taichui* are nearly the same shape as those of *Monorchotrema taihokui,* but their color is a lighter yellow and somewhat brighter. The size is also somewhat smaller, i. e., the average length is 21.4μ (20.7 to 23μ) and the average width, 10.6μ (9.2 to 10.7μ). The shape is symmetrically ovoid (Figs. 7, 8,; Textfig. 2). The front portion has an operculum. The shoulder thickening is inconspicuous. The posterior end is thickened and forms a protuberance similar to that of Clonorchis eggs.

The eggs of *Clonorchis sinensis* resemble those of *Monorchotrema taihokui* in size and shape. These two are difficult to discriminate. The shoulder of the latter is broader and less prominently sculptured. *Monorchotrema taichui* has an egg shell structurally like that of *Clonorchis sinensis,* but has a much less conspicuous shoulder. *Monorchotrema taichui* is slightly smaller than that of *Clonorchis sinensis.* The eggs of *Metagonimus yokogawai* bear a general resemblance to those of *Monorchotrema taihokui* and *M. taichui,* but there is no thickening or shoulder at the boundary between the operculum and the shell in the case of Metagonimus. Also the eggs of *Metagonimus yokogawai* are slightly larger than those of the two Monorchotrema species, and are considerably broader.

The eggs of the two Monorchotrema species are easily distinguished from those of *Stamnosoma formosanum* Nishigori and *Stamnosoma armatum* Tanabe, since the larvae within the egg-shell of the latter two forms are always immature, while the metraterm eggs of both of the Monorchotrema species contain mature miracidia; also in *Stamnosoma formosanum,* the egg-shell possesses a latticed or fretted design, which has not been seen in the Monorchotrema forms.

While differentiation of the eggs of these several species of flukes is entirely possible, careful study is required. It seems probable that errors have frequently been made by clinicians who have been familiar with only one kind of the several species of eggs in question and have not been aware of the need for extremely careful comparison of distinct but closely related characters.

TABLE 13.—*Measurement of the Eggs of Monorchotrema taihokui*

Length (μ)	Breadth (μ)
27.5	15.3
29.0	12.9
26.1	15.3
27.5	13.8
27.5	12.3
27.5	12.3
26.0	12.3
29.0	13.8
26.0	12.2
24.5	15.3
Average, 27.1μ	Average, 13.5μ

TABLE 14.—*Measurement of the Eggs of Monorchotrema taichui*

Length (μ)	Breadth (μ)
20.7	10.7
21.4	10.7
21.4	10.7
21.4	10.7
22.2	9.2
21.4	10.7
20.7	10.7
23.0	10.7
21.4	10.7
23.0	10.7
Average, 21.4μ	Average, 10.6μ

TABLE 15.—*The Measurement of the Eggs of Monorchotrema taihokui and M. taichui and the Eggs of Other Flukes, With Which They Might Be Confused*

Family	Egg	Length (μ)	Diameter (μ)	Measured by
Opisthorchiidae	Opisthorchis felineus	30	12	Looss (1905)
Opisthorchiidae	Clonorchis sinensis	26–30	15–17	Kobayashi (1917)
Heterophyidae	Metagonimus yokogawai	27–30	15–17	Yokogawa (1913)
Heterophyidae	Heterophyes heterophyes (velnocens)	27–29	15–16	Onji and Nishio (1915)
Heterophyidae	Stamnosoma armatum	28–33	16–17	Tanabe (1922)
Heterophyidae	Stamnosoma formosanum	33–35	17–20	Nishigori (1924)
Heterophyidae	Monorchotrema taihokui	26.1–29	12–15	Faust and Nishigori (this study)
Heterophyidae	Monorchotrema taichui	20.7–23	11	Faust and Nishigori (this study)

THE CERCARIAE OF THE FAMILY HETEROPHYIDAE

With the knowledge of the life cycles of three genera of the Heterophyidae, including the description of the cercariae of five species (viz., *Metagonimus yokogawai, S. formosanum, Monorchotrema taihokui* and *M. taichui*) as a background, an opportunity is afforded for comparison and differentiation of the characteristics of this larval stage in the family under consideration. In addition to these cercariae certain others which have been described from the Far East undoubtedly belong to this group. Among others, attention is called to *Cercaria photifera* Faust 1922 and *Cercaria cordata* Faust 1924. Two other species, as

yet undescribed, which have been studied by one of us (Faust) are now added to the series.

Cercaria translucens nov. spec.
(Fig. 23)

This cercaria was found in a collection of *Bithynia striatula* collected near Chaochowfu, some twenty-five miles above Swatow, Kwangtung Province, China. The snails were removed from water and allowed to dry out, in order that they might be removed to Peking alive. On placing the snails in water some four weeks later, the majority of them soon began to revive and for several days after this cercariae were found at intervals in the water. Dissection of some of the snails also revealed them in the interhepatic lymph spaces. This cercaria, for which we propose the name *C. translucens,* is a larva with an oval body, rounded at the posterior end and gently tapering anteriorly (Fig. 23). A short distance behind the oral sucker is a pair of pigmented eye-spots (*es*), situated on the dorsal side far out toward the lateral margins of the fluke. The retina is cup-shaped and the lens lies within the concavity. The entire body is slightly pigmented but most of the pigment granules are clumped around the eye-spots. The entire body is covered with small but conspicuous spines (Fig. 23 *a*) which are heavier and larger in the immediate vicinity of the oral opening. Immediately surrounding the oral aperture is a crown of spines, each one of which consists of a sharp tooth and two sharp roots (Fig. 23 *b*). The tail is stout and muscular and is provided with a conspicuous fluted keel on both lateral margins. The keel is transparent and is seen only under subdued light. When the tail is even slightly contracted as in figure 23, the tissue of the keel overlaps in such a way as to give a pleated appearance. These points of overlapping frequently give the impression of delicate spinose projections from the tail trunk. The tail is, however, entirely aspinose.

The body has an average length measurement in formalin-fixed speciments of 0.45 mm., and an average width of 0.225 mm. The tail has a length of 0.675 mm. when relaxed but may reach 0.9 mm. on extension. It measures 27μ in cross-section at its base. The tail fins are as wide as the trunk of the tail. The caudal body is about 30μ thick. The oral sucker of the cercaria has a width of 36μ and a depth of about 40μ. It is directed somewhat ventrad. The ventral sucker lies in the midplane on the ventral side of the body. It has an outer portion with a diameter of 24μ and an inner one of 13.6μ. No details of the digestive tract have been observed either in living or stained specimens. The cephalic secretory glands (*cg*) consist of six pairs of unicellular organs situated on either side of the acetabulum. They appear to be slightly oxyphilic in reaction. The proximal ends of their ducts (*cgd*) are enlarged but the distal ends become constricted as they approach the

anterior margin of the larva and emerge through minute capillary openings on the dorsal wall of the oral sucker. The excretory system consists of a large, transversely compressed bladder (*eb*) and two main collecting tubes (*et*) with dilated proximal portions and constricted anterior portions. On reaching the plane of the eye-spots these tubules suddenly bend backward and become lost in the glandular mesenchymatous tissue. The reservoir is filled with highly refractive excretory concretions of various sizes. No tubule has been found in the tail. In stained specimens the genital mass (*ga*) is seen as one clump of large cells immediately behind the acetabulum and above the pharynx.

Cercaria translucens develops in a redia which cannot be differentiated from the rediae of other members of the family. The cercaria is very active when it first emerges from the snail. It will swim about energetically for several hours. After a while, however, it becomes quiescent, having apparently exhausted its reserve energy. It discards its tail and forms a cyst capsule which has been secreted by the cystogenous cells lying loosely under the integument. In the event that cyprinoid fishes are placed in the aquarium with specimens of *Bithynia striatula* discharging these cercariae, the cysts will be found in the course of twenty-four hours to have encysted under the scales of the fish. The cysts can be distinguished only with difficulty from those of Metagonimus, Heterophyes, and those of the two species of Monorchotrema.

Cercaria tridonta nov. spec.

Cercaria tridonta (Fig. 24) was found as a light infection in two consignments of *Bithynia sinensis* forwarded to one of us (Faust) in Peking during the fall of 1924, by Dr. H. B. Taylor of Anking, Anhwei Province, China, who collected them from bathing pools near the city of Anking. The snails were in good condition on examination three weeks after posting, and dissection of several revealed light infections with this larval form. In some respects this cercaria resembles *C. translucens*. In many ways, however, it is more like *C. photifera* from *Viviparus polyzonatus,* collected by one of us (Faust) in 1921 at Wuchang, Hupeh Province, China. *C. tridonta* is somewhat smaller than *C. translucens,* with a body measuring 0.35 mm. in breadth, and with a tail having a length nearly twice that of the body and a cross-section measurement of 21μ. The tail is keeled similarly to that of *C. translucens,* and the eye-spots in the anterior region of the body are situated in a similar position to those of the latter species. The body integument is provided with a spinose armament, the anteriormost portion of which consist of a crown of sharp spines (Fig. 24 *a*), each having a root, a vertical shaft and a lateral arm. The shape of the relaxed body of *C. tridonta* is pyriform. The bladder and collecting tubules of the

excretory system are like those organs in *C. translucens*. Instead of six pairs of oxyphilic cephalic glands there are from sixteen to twenty pairs of these glands with mucoid contents. The oral sucker has a spherical outline and a diameter of 27μ. The ventral sucker has a cross-section measurement of 21μ. It lies somewhat behind the middle of the body. No genital mass has been observed. The parthenita is a redia with the characteristics of other rediae of the group. The phenomena of movement, encystment and the utilization of a fish as secondary intermediate host are all similar to those described for *C. translucens*.

In comparing the cercariae of this group with one another we find common resemblances, consisting of spinose integument, a tail with lateral fluted keels, and pigmented eye-spots, while all seven of the cases where data are available show the same type of transfer from the mollusc to the definitive host, namely, by encysting on or in the tissues of fresh-water fishes, from which they are taken into the digestive tract of the final host. It is much more illuminating, however, to regard these larvae as belonging to a well-graded series, of which *Cercaria cordata* stands at one end and the cercaria of *Metagonimus yokogawai* stands at the other. From the viewpoint of larval characters the cercarie of *S. formosanum* (Nishigori, 1924; fig. 2) are more closely related to *C. cordata* and *C. photifera* than to the others, while the cercariae of the Monorchotrema species (Figs. 14, 20) are nearer to the cercaria of Metagonimus. The two new cercariae, *C. tridonta* and *C. translucens* are intermediate in the series. While similarities exist among the members of this group it is well to keep in mind, when attempting to differentiate them from other oculate lophocercous cercariae, that they form a well-graded series and not a single type.

DISCUSSION OF LIFE CYCLE

Upon analyzing the data obtained from the study of the two species of the genus Monorchotrema and of the two larval forms, *Cercaria translucens* and *C. tridonta,* the type of life cycle previously described for Metagonimus (Yokogawa, 1913) and for Stamnosoma (Nishigori, 1924) is found to be a constant character for the known members of the family. Furthermore, data obtained by Onji and Nishio (1915) for Heterophyes in Japan, by Kobayashi (1924) and Khalil (1924) for Heterophyes in Egypt and by Ciurea (1924) for Apophallus, which belongs to an entirely different subfamily of the Heterophyidae, show the existence of an identical host relationship, at least as far as the fish-source of infection is concerned. It is highly probable that all members of the family Heterophyidae follow the same essential cycle of infection, namely, from definite host to mollusc, from mollusc to fresh-water fish, and from fresh-water fish to definitive host again. Furthermore, although the miracidia and the cercariae have common character-

istics, just as do the adult worms of the group, subfamily and probably generic differences in these two stages of larvae can undoubtedly be recognized, even though these differences may not be correlated *ad seriatim* with the gradational differences of the adult forms.

Most helminthologists have followed Looss's view (1899) that although flukes found in birds and in mammals may be morphologically indistinguishable, they should be recognized as physiologically different species or varieties, due to habitat and geographical distribution, presumably provoked by the metabolic differences in these two groups of hosts. However, Looss never put his theory to the experimental test. In two instances at least (*Stamnosoma formosanum* and *Monorchotrema taihokui*), found naturally as adults in the night-heron, the life cycle has been experimentally completed in various mammals including man. In the case of *M. taihokui* the experimental infection was initiated with ova obtained from the bird host and completed in mammal which were not previously known to be susceptible to the infection. Even if this had not been the case the discovery of the adult flukes first in birds and later introduced experimentally into mammals in the same locality would have been strong presumptive evidence against Looss's hypothesis. Such evidence is also obtainable from the data of Ciurea (1924) on *Apophallus mühlingi,* found as a natural infection in *Larus ridibundus* and introduced experimentally into the dog. Thus we find that in the family Heterophyidae evidence preponderates in favor of the view that the adult worm may live in both birds and mammals. There appears to be no need, therefore, to create specific or varietal names for members of this family which are morphologically indistinguishable, even though they are found in birds on the one hand and in mammals on the other.

The evidence stated above brings us to a consideration of the form *Centrocestus cuspidatus* var. *caninus* Leiper, 1913, named by Leiper from material from Formosan dogs furnished by Dr. Yokogawa. The question is not an easy one to settle because of the imperfect preservation of the specimen examined by Leiper. In view of the foregoing evidence it is possible to assume one of two points of view. (1) The specimen in question, which Leiper was not able to distinguish from *Centrocestus cuspidatus* Looss, obtained from *Milvus parasiticus* in Egypt, should not, in view of our present knowledge, be recognized as a distinct variety merely because it was found in a mammal. (2) Since the specimen was obtained from the dog in the same locality where *Stamnosoma formosanum* has been experimentally developed in the dog, and since there are no fundamental differences in structure between Leiper's specimen and *S. formosanum,* this form should be regarded as synonomous with the latter species. In case the second conclusion is maintained the validity of the genus Stamnosoma must be considered. *S. formosanum* differs from *Centrocestus cuspidatus* (Looss, 1896: 97) in having a much less

extensive prepharynx, in having a well-developed esophagus (*C. cuspidatus* has none), in possessing lobate testes and ovary, and in having a more particulate distribution of the vitellaria than *C. cuspidatus*. With respect to the first question, Looss's figure (1896, fig. 65) shows definite prepharyngeal outpocketings, which Stamnosoma does not possess. Viewing the problem as a whole we favor the recognition of Stamnosoma as a valid genus and are inclined to regard *Centrocentus cuspidatus* var. *caninus* Leiper, 1913, as a specimen of *S. formosanum,* since in our opinion, Leiper's sketch more closely resembles *S. formosanum* than it does Centrocestus Looss.

With respect to *Pygidiopsis genata,* found by Looss in the pelican at Cairo (1907) and by one of us (Faust) in the dog at Canton, we feel that we are dealing with the same species in both hosts. Careful examination of our material shows it to agree in every respect with the description and figures published by Looss. The species in question must be regarded as a facultative parasite of both mammals and birds. Our material is generically different from Ascocotyle just as that of Looss, in the absence of circum-oral spines and of the oral cecum and does not admit, therefore, of the suggestion made by Ransom (1920: 570) that this species, *P. genata,* might be found on further examination to belong to the genus Ascocotyle.

Species of the genus Melania are the most usual first intermediate hosts of heterophyid infections. However, at times, species of Bithynia and even of Viviparus, and possibly of other operculate fresh-water molluscs may serve in this capacity.

All the data in hand point to the belief that although the encysted forms usually occur in cyprinoid fishes, any fresh-water fish found in the appropriate habitat may serve as the second intermediate host.

KNOWN SPECIES OF HETEROPHYIDAE IN THE FAR EAST

The following forms belonging to this family have been found in the Far East:

1. *Heterophyes heterophyes* (v. Siebold 1852).
   Synonyms: *Distoma heterophyes* v. Siebold 1852.
   Distoma heterophyes hominis Diesing 1855.
   Dicrocoelium heterophyes Weinland 1858.
   Fasciola heterophyes Moquin-Tandon 1860.
   Heterophyes aegyptiaca Cobbold 1866.
   Mesogonimus heterophyes Railliet 1890.
   Coenogonimus hererophyes Looss 1899.
   Cotylogonimus heterophyes Lühe 1899.
   Heterophyes nocens Onji and Nishio 1915.
   Heterophyes nocens (of Onji and Nishio 1915) Cort and Yokogawa 1922.

Definitive Hosts:
Man, dog, cat, fox (?).
Localities: Egypt, Japan, Central and South China, Korea, Formosa.

2. *Metagonimus yokogawai* (Katsurada) June 30, 1921. First described as *Heterophyes yokogawai* Kats., May 31, 1912, *Loxotrema ovatum* Kobayashi Oct. 10, 1912. [*Loxotrema* preocc. *Loxotrema* Gabb 1868 *Mollusca,* Am. Jour. Couch., 4: 147] [*Metagonimus ovatus* Yokogawa 1913, although originally intended to designate a different species, is also synonymous with *M. yokogawai* (Kat.)].
Synonyms: *Heterophyes yokogawai* Katsurada 1912.
Loxotrema ovatum Kobayashi 1912.
Metagonimus yokogawai Katsurada 1912.
Tocotrema yokogawai Katsurada 1912.
Metagonimus ovatus Yokogawa 1913.
Yokogawa yokogawai Leiper 1913.
Loossia romanica Ciurea 1915.
Loossia parva Ciurea 1915.
Loossia dobrogiensis ciurea 1915.
Definitive Hosts:
Man, dog, cat, pig, mouse, etc., pelican (*Pelicanus onocrotalus*).
Localities: Japan, Central and South China, Korea, Formosa, Roumania.

3. *Monorchotrema taihokui* Nishigori 1924.
Definitive Hosts:
Night-heron (*Nycticorax nycticorax*), man, dog, cat, etc.
Locality: Formosa.

4. *Monorchotrema taichui* Nishigori 1924.
Definitive Hosts:
Experimental mammals.
Locality:
Formosa.

5. *Ascocotyle pithecophagicola* (Faust) 1920.
Synonym: *Phagicola pithecophagicola* Faust 1920.
Definitive Host:
Monkey-eating eagle (*Pithecophaga jefferyi*).
Locality: Philippine Islands.

6. *Stamnosoma armatum* Tanabe 1922.
Definitive Host:
Night-heron (*Nycticorax nycticorax*); man and other mammals.
Locality: Japan.

7. *Stamnosoma formosanum* Nishigori 1924.
   Synonym (?):
   Centrocestus cuspidatus var. *caninus* Leiper 1913.
   Definitive Hosts:
   Night-heron (*Nycticorax nycticorax*), man, dog, cat, etc.
   Locality: Formosa.
8. *Pygidiopsis genata* Looss 1907.
   Definitive Host:
   Dog.
   Locality: Canton, 1923.

LARVAL FORMS

1. *Cercaria translucens* n. sp., from *Bithynia striatula.*
   Locality: Near Swatow, Kwangtung Province, China.
2. *Cercaria tridonta* n. sp., from *Bithynia sinensis.*
   Locality: Anking, Anhwei Province, China.
3. *Cercaria photifera* Faust 1922, from *Viviparus polyzonatus.*
   Locality: Wuchang, Hupeh Province, China.
4. *Cercaria cordata* Faust 1924, from *Melanoides tuberculatus.*
   Locality: Canton, Kwangtung Province, China.
5. *Cercaria chromophila* Faust 1922, from *Melania ebenina.*
   Locality: Kiukiang, Kiangsi Province, China.

SUBFAMILY GROUPS IN THE FAMILY HETEROPHYIDAE

Of the five subfamilies designated by Ciurea (1924) for the family Heterophyidae under consideration, three occur in the Far East. The subfamily Monorchotreminae Nishigori 1924 is now added to the ones previously designated to include the two species which have formed the basis of this study.

Subfamily Monorchotreminae Nishigori 1924. Heterophyidae distinguished by the presence of a single large conspicuous testis in place of the two testes found in all previously described species of this family. Genital sucker fused in part with the ventral sucker, surrounded by a half-circlet of genital hooklets. Definitive hosts: birds and mammals, including man. Represented by a single described genus, Monorchotrema. Described species, *Monorchotrema taihokui* and *M. taichui.* Locality: Formosa.

SUMMARY

1. Two new heterophyid flukes, *Monorchotrema taihokui* and *M. taichui* from Formosa differ from previously described species of the family in having only a single testis.

2. *Monorchotrema taihokui* has been found as a natural infection in the night-heron, *Nycticorax nycticorax,* and in the dog and cat, and as an experimental infection in various mammals, including man. *M. taichui* develops experimentally in mammals and probably also in birds. It also occurs as a natural infection in the cat.

3. Melaniid gasteropods (*Melania reiniana* var. *hidachiens* and *M. obliquegranosa*) are the first intermediate host and various fresh-water fishes the second intermediate hosts. The cercariae encyst on the cartilaginous tissues of the fins and head and on the gills. Infection is incurred by the definitive host by consumption of the raw infected fishes.

4. The life cycle of the species is described and compared with related forms.

5. The eggs of *Monorchotrema taihokui* and *M. taichui* are described and criteria are presented for differentiating them from one another and from similar eggs of other flukes.

6. Two new species of cercariae from China, *C. translucens* and *C. tridonta,* belonging to the Heterophyidae are described. The relationship of the cercariae of the family is discussed. The known species form a well-graded series and not a single type.

7. All members of the family Heterophyidae have a common type of life cycle, involving an operculate snail, a fresh-water fish and warm-blooded vertebrates. Both birds and mammals may harbor the definitive stage of the same species, apparently equally well.

We wish to express our most sincere thanks and gratitude to Professor S. Yokogawa, for his suggestions and assistance during the progress of this study, to Professor H. Kobayashi for the privilege of examining cotype specimens of *Loxotrema ovatum* Kobayashi 1912, and to Professor Ciurea for presentation of a number of Heterophyidae from the Near East.

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EXPLANATION OF PLATES

c cecum
ca tail
cg cephalic gland
cgd cephalic gland duct
cgo cephalic gland duct opening
e esophagus
eb excretory bladder
es eye-spot
et excretary tubule
ga genital or germinal mass
gp genital pore
gs genital sucker
oo oral opening
os oral sucker
ov ovary
p pharynx
pp prepharynx
sg secretory glands
sgd secretory gland duct
sr seminal receptacle
sv seminal vesicle
t testis
ve vas efferens
vit vitellaria
vs ventral sucker

Explanation of Plate III

Fig. 1.—Ventral view of adult *Monorchotrema taihokui.* × 140.

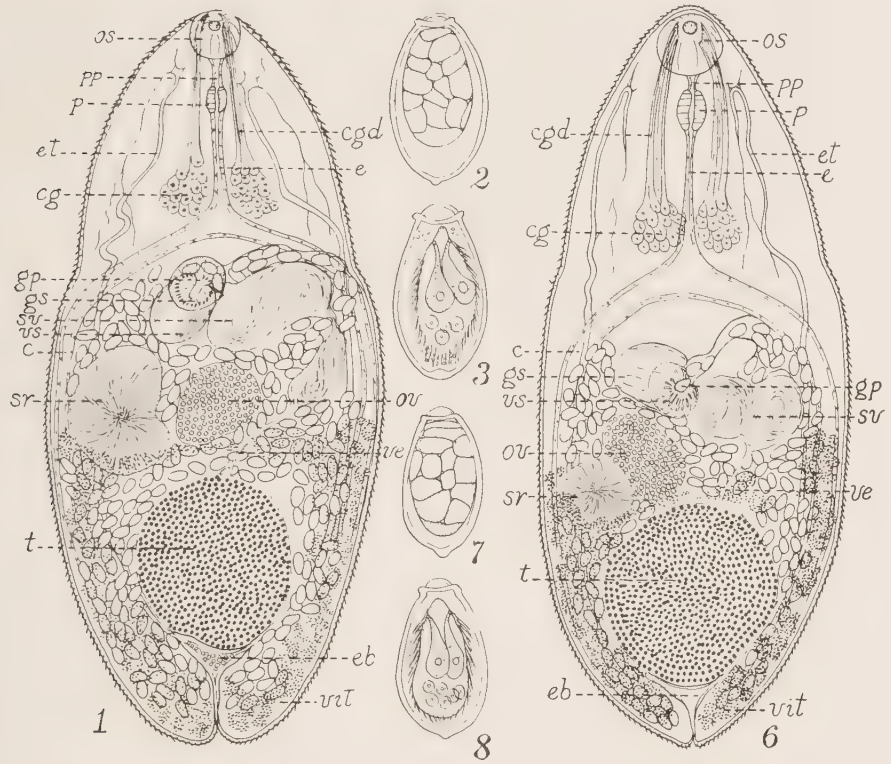
Figs. 2, 3.—Eggs of *M. taihokui;* fig. 2, immature; fig. 3, mature. × 890.

Figs. 4, 5.—Detail of "genital-ventral-sucker-apparatus;" fig. 4, frontal view; fig. 5, sagittal section. × 280.

Fig. 6.—Ventral view of adult *Monorchotrema taichui.* × 140.

Figs. 7, 8.—Eggs of *M. taichui;* fig. 7, immature; fig. 8, mature. × 890.

Figs. 9-11.—Detail of the "genital-ventral-sucker-apparatus;" fig. 9, ventral view; fig. 10, sagittal section; fig. 11, oblique section. Fig. 9, × 240; figs, 10, 11, × 200.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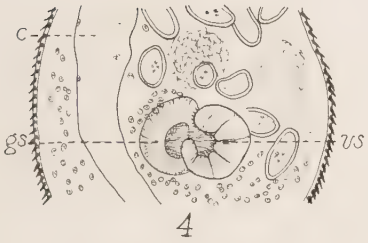
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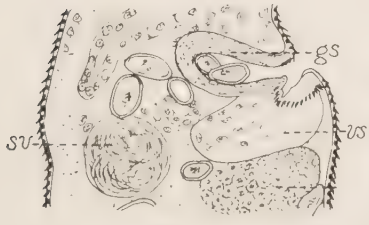
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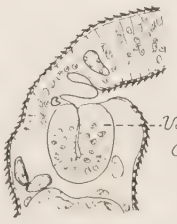
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PLATE III

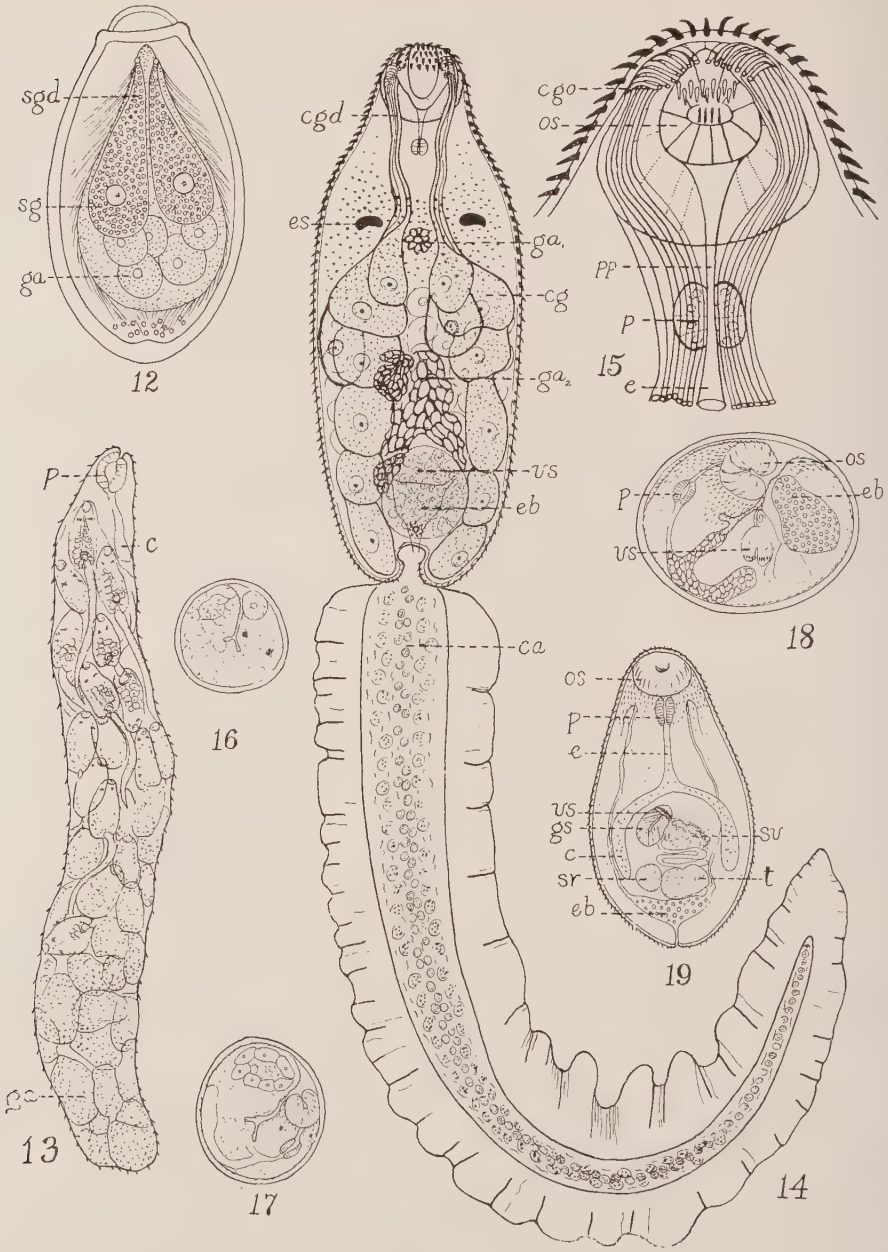

PLATE IV

EXPLANATION OF PLATE IV

Fig. 12.—Egg of *Monorchotrema taikohuki,* showing mature miracidium. × 1,800.

Fig. 13.—Young redia of *M. taihokui,* with developing cercariae. × 80.

Fig. 14.—Cercaria of *M. taihokui,* ventral view. × 300.

Fig. 15.—Anterior end of the cercaria of *M. taihokui,* showing relation of cephalic duct opening to oral sucker. × 900.

Figs. 16-18.—Stages in development of the encysted adolescaria of *M. taihokui;* fig. 16, 6 days after encystment; fig. 17, 7 days after encystment; fig. 18, 19 days after encystment. × 160.

Fig. 19.—Young adolescaria from the intestinal mucosa of the definitive host (dog), 2 days after experimental feeding. × 133.

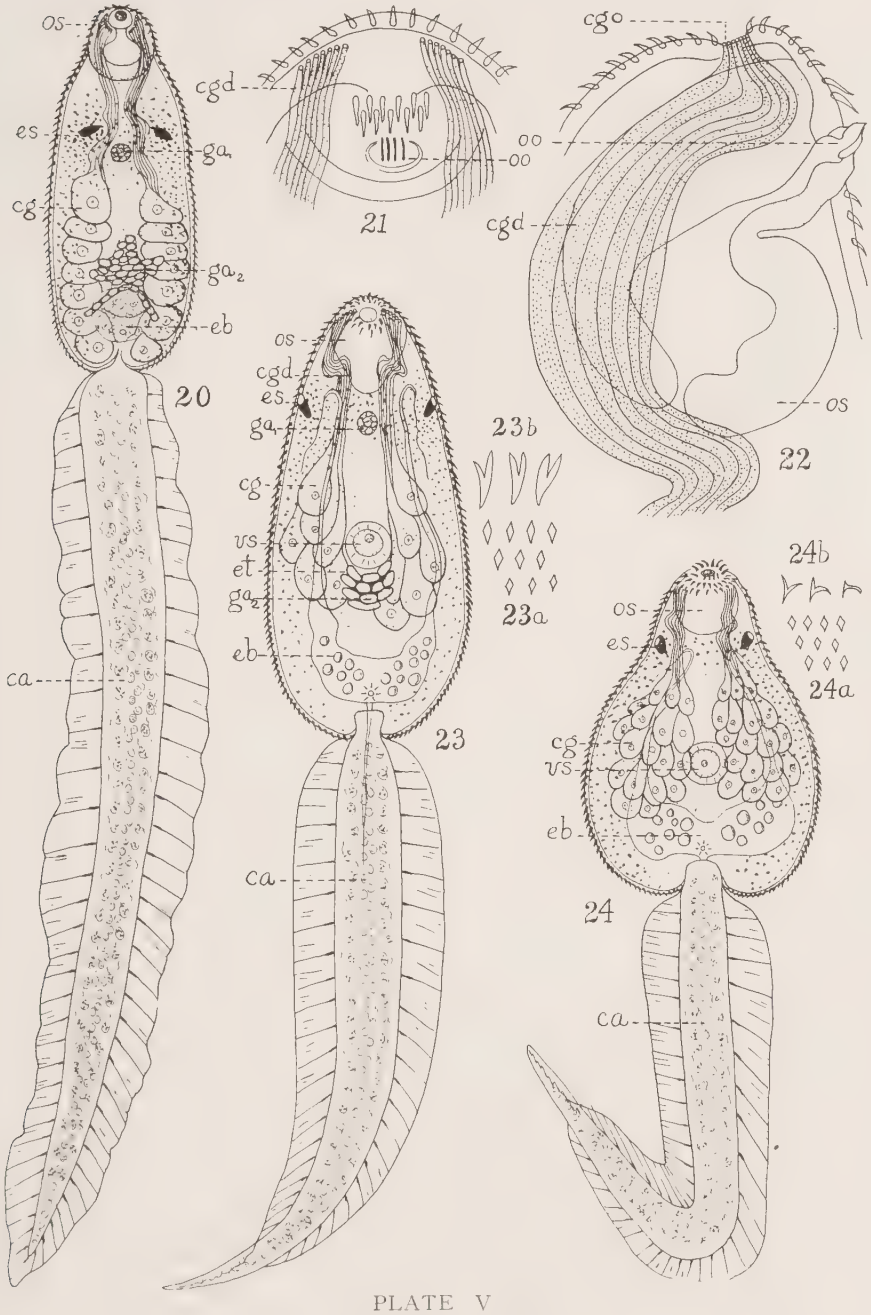

PLATE V

Explanation of Plate V

Fig 20.—Cercaria of *M. taichui,* ventral view. × 300.

Figs, 21, 22.—Oral and lateral views of anterior end of the cercaria of *M. taichui,* showing "invasion apparatus" of the cercaria. × 900.

Fig. 23.—*Cercaria translucens,* ventral view. × 133. Fig. 23a, body spines; fig. 23b, oral spines; greatly enlarged.

Fig. 24.—*Cercaria tridonta,* ventral view. × 133. Fig. 24a, body spines; fig. 24b, oral spines; greatly enlarged.

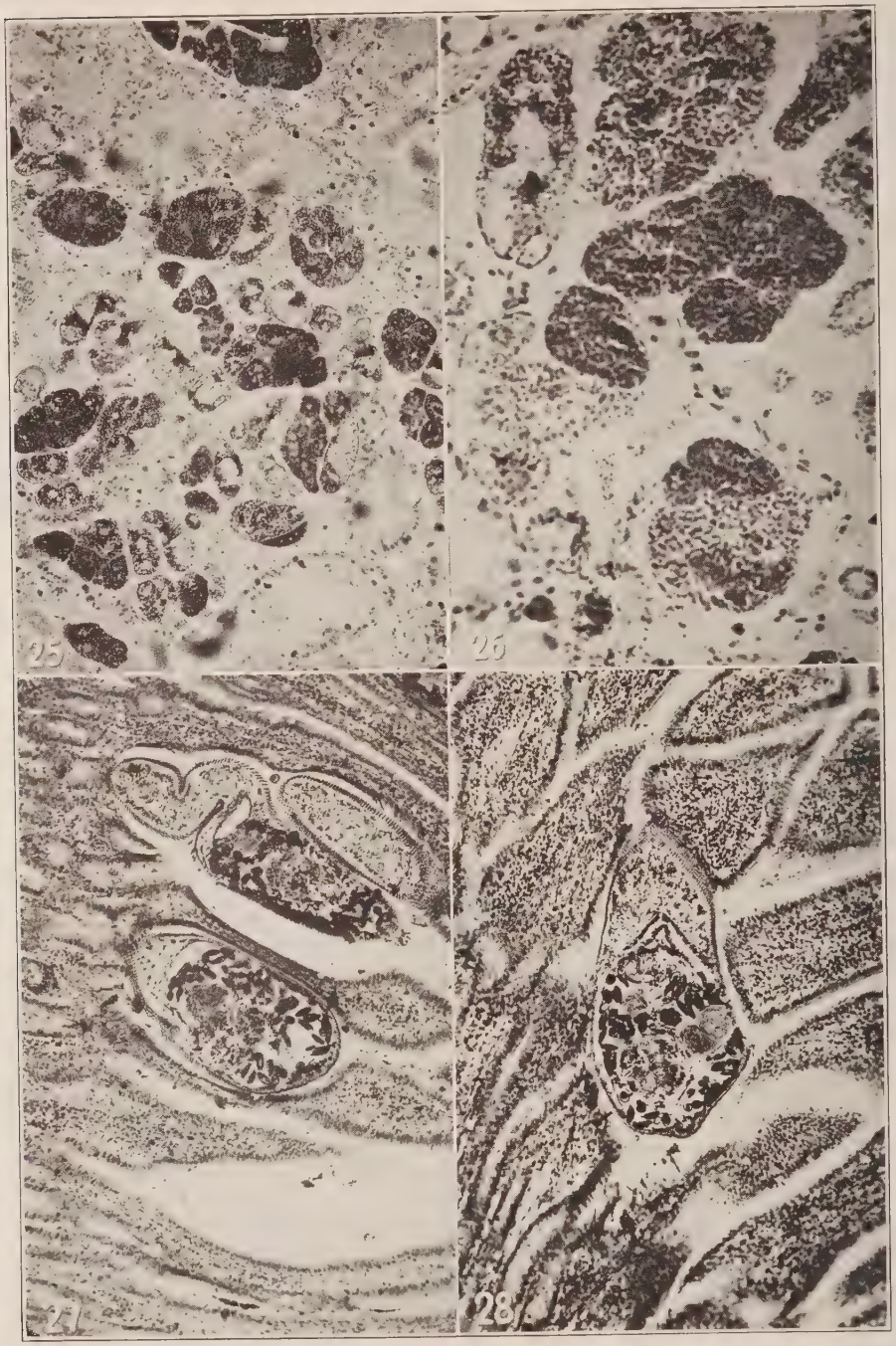

PLATE VI

Explanation of Plate VI

Fig. 25.—Photomicrograph of the rediae and cercariae of *M. taihokui* in the inter-hepatic lymph spaces of *Melania reiniana*, var. *hidachiens*. × 100.

Fig. 26.—Similar photomicrograph of *M. taichui* in *Melania obliquegranosa*. × 250.

Fig. 27.—Photomicrograph of adult *M. taihokui* attached to the intestinal wall of the definitive host (experimental dog). × 100.

Fig. 28.—Similar photomicrograph of *M. taichui* in the dog's intestine. × 100.

ON THE BIOLOGY AND LIFE HISTORY OF *RHODNIUS PROLIXUS* STAHL*

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The importance of *Rhodnius prolixus* in human pathology was shown by Brumpt (1913) when he obtained experimental infections with *Trypanosoma cruzi* in larvae and adults raised in his laboratory from eggs sent him from Venezuela. Tejera (1919) working in the Maracaibo Lake basin found several specimens of *R. prolixus* harboring Trypanosoma forms morphologically identical with *T. cruzi* and succeeded in the inoculation of small rodents. This same investigator found flagellated forms of *T. cruzi* in the blood of patients from the state of Zulia. In our laboratory several hundred specimens of *R. prolixus* in different stages of development have been examined and the percentage of flagellate infection has been found to be very high (1922).

Rhodnius prolixus is known in the state of Trujillo, Venezuela, by the name of "Pito" (whistle), and as "Chupon" (sucker) in the states of Merida and Tachira. In other localities it is known by the names of "Chipo" and "Chinche de monte" (forest bug). The name of "Pito" seems most inappropriate, for although the natives claim that when "Pitos" fly, some sort of whistling noise is heard, I have frequently observed *Rhodnius* in flight and the motion of the wings apparently did not produce sounds of the type in question.

The area of distribution of *R. prolixus* in the state of Trujillo is very wide. Our laboratory received specimens from the Maracaibo Lake Shore and from several small towns and villages located on the foot hills of the western slope of the Venezuelan Andes, up to an alittude of 1,300 meters above sea level. The largest lots were received from the towns of Carvajal, Pie-de-Sabana, Mendoza, and San Jacinto. The last two towns mentioned are well known in Trujillo for the prevalence of endemic goiter, pseudo-myxedema, and the number of cretins and undeveloped children. In the other two towns, however, goiter and cretinism is quite rare, though large numbers of Rhodnius infest almost every house.

Smaller lots were received from numerous farm houses located on the hills nearby the above mentioned towns, as well as from farms surrounding the city of Trujillo, the capital of the state. The presence of Rhodnius was reported from numerous places in and outside the

* Investigation carried out in the Laboratory of the Venezuela Sun Company, Valera, Venezuela.

state of Trujillo. It seems that Rhodnius is more frequently found in a zone situated between sea level and an altitude of 1,300 meters. It was observed that the latter represents roughly the upper limit of the infested zone, although there were a few places slightly higher from which mild infestation with Rhodnius was reported. A certain degree of moisture and heat, together with good hiding places, such as are provided by the primitive construction of the houses in the lower tropical countries, are the main requisites for its presence.

Rhodnius prolixus, as far as we know, is strictly domiciliary in habit, although it is possible that this mode of life has been somewhat recently acquired. Adult females were kept in our laboratory in glass jars to which green leaves and strips of paper were added, and it was later found that eggs were laid on the leaves, agglutinated in the usual way observed in others kept with paper strips only. Natives believe that the insects are brought in on the palm leaves used for roofing, and showed us some insects on the newly collected palm leaves which were not even related to Rhodnius. Its presence in palm thatched houses or shacks is due to the fact that most adequate shelter is furnished in the interstices of the roof as well as in the cracks of the mud walls of these primitive dwellings.

In such places, where "Pitos" are so numerous, one wonders how people can live among swarms of such reputably dangerous insects, and still appear to be in good health, as good health goes in this climate. Such houses are sometimes abandoned on account of the Pito nuisance but not on account of any suspected endangerment to health.

Triatoma megistus Burmeister is said to live in burrows made by the Armadillo (*Dasypus novemcinctus*), an animal which has been reported to serve as a reservoir for *T. cruzi.* Tejera (1919) reports the same fact for *Rhodnius prolixus,* which as stated by certain native observers has been found not only in burrows made by armadillos but also in the burrows made by a large rodent locally called "Lapa" (*Coelogenys subniger*). I have repeatedly asked the native hunters among whom there are several intelligent observers who would readily recognize the "Pito," but, even for two years of searching for them in burrows made by various sorts of wild animals, their reports have been always negative.

The question raised by the report of Tejera is of great importance, due to the possible identity of the human Trypanosoma and the ones found in the blood or other tissues of burrowing animals. I have examined the blood of numerous Armadillos and have made sections of different tissues from several young and adult specimens, but the results have been always negative. I also tried to keep some in semi-captivity and to infect them with intestinal contents of *Rhodnius prolixus* rich in Trypanosoma forms showing the morphology of *T. cruzi,* but it was

impossible to keep them very long, for those that did not escape from the garden, died before any results were obtained.

The domestic habits of *Rhodnius prolixus* are very similar to those described for *Triatoma infestans* in Brazil, Argentine, and Chile (Klugg, 1908; and Poeppig, E., 1835). "Pitos" are abundant in all badly kept shacks, where innumerable cracks in the mud walls, as well as the interstices of the thatched roofs afford excellent hiding places. They hide during the day and come out at night to feed upon human blood as well as on that of any domesticated animal, numbers of which live promiscuously with the careless dwellers. The adult Rhodnius customarily bites any exposed part of the body and as it flies readily, persons sleeping in hammocks are also subject to their attacks. Larvae and nymphae feed especially on persons sleeping on beds close to the walls, but they may also crawl up the legs of the beds.

When a house is deserted, the insects migrate to another locality, being able to fly a considerable distance. Mule packs serve very well as a means of transportation for the insects, as they can crawl into the innumerable hiding places afforded by pack saddles and cargoes.

The bite is nearly painless as I know from personal experience in feeding insects kept in the laboratory as well as from the reports of reliable persons. I was usually bitten on the tip of the finger where sensibility is pronounced, and still I believe that even here there was not sufficient pain to disturb a person when sound asleep. Though the bite is almost painless, many people among the natives assure one that it is very painful and is followed by considerable swelling of the bitten part, and some go so far as to believe that ulcers called "Pito bite" (which are clearly Leishmania lesions) are produced by the bite of Rhodnius. The bite of *Triatoma infestans* Burmeister is said by Fairmaire (1876) to be rather painful, producing considerable swelling of the bitten part, but Neiva (1913) when speaking of the "Vinchuca" (*T. infestans*) states that its bite is almost painless. All of the insects hatched in the laboratory were fed by allowing them to suck blood from our fingers In a few instances a slight induration of the skin at the place of the bite was observed which lasted for a few days and then completely disappeared, but in no case was there any ill effect experienced.

It seems convenient to quote what Darwin (1845) says when speaking of the "Vinchuca."

"When placed on a table and though surrounded by people, if a finger was presented, the bold insect would immediately protrude its sucker, make a charge and, if allowed, draw blood. No pain was caused by the wound. It was curious to watch its body during the act of sucking, as in less than ten minutes it changed from being as flat as a wafer to a globular form. This one feast, for which the venchuca was indebted to one of the officers, kept it fat during four whole months; but, after the first fortnight, it was quite ready to have another suck."

LABORATORY OBSERVATIONS

Adult females were secured from numerous heavily infested shacks near the town of Valera, Venezuela, and kept in separate, wide-mouth glass bottles, capped with wire cloth. Strips of filter paper were put in the bottles so as to afford resting places for the insect as well as to receive the abundant fecal material which it passes. By merely changing the dirty strips for new ones, comparatively clean quarters were furnished the stock on hand. Under such conditions, it was observed that the insects rested on the side of the paper which was less exposed to the rather bright light of the laboratory. When the bottle was turned, exposing them to the light, they remained motionless for some hours but finally moved around to the opposite side. If not disturbed, they remained indefinitely on the shaded side, making only occasional excursions along the paper strips and on the wire-cloth cap.

The eggs (figs. 1-4) are elongated, measuring 2.5 mm. in length, rose color when recently laid, turning to bright lobster-red after a few hours. One of the extremities shows a marked constriction forming a short flask neck, and is closed by a thin, whitish operculum. The cuticula of the egg is granular and slightly resistant so that unless in a moist atmosphere the embryo dries up and the shell wrinkles and collapses. The eggs are laid in small lots ranging from 1 to 14, scattered over the filter paper or agglutinated in alternating rows in which the non-operculated end of the egg looks toward the middle of the row (fig. 2). On several occasions, the insect protruded the ovipositor through the meshes of the wire-cloth, and laid several eggs on the outside. Small card-board boxes with narrow slits were put in some of the bottles, and it was observed that numerous eggs were deposited on the innermost border of the slit. This habit of laying the eggs in cracks and small holes tends to afford protection from outside dangers as well as from excessive light and dry heat.

The total number of eggs varied from 200 to 300 from one single female, laid in 30 to 50 batches of which the first ones were more abundant, ranging from 8 to 14 eggs each. It was observed that the number of batches of eggs laid as well as the number of eggs in each increased on augmentation of either the number of feedings or the amount of food taken. After six to seven days of incubation, the eggs if fertile showed two black spots near the operculated end. Fresh laid eggs kept at room temperature ranging from 27 to 32.5 C. hatched on the twelfth day of incubation. When kept on the top of an incubator at a temperature of about 34 C., they hatched in ten to eleven days. Kept in the incubator at 37 C., even if sufficient moisture was provided, most of them died in the first three to four days, while none of the others ever hatched unless soon removed and placed at room temperature.

The newly hatched larvae (fig. 6) measure 2.7 mm. in length, and are of a bright lobster color but turn to dark brown in the course of 15 to 30 minutes. They are very lively, resembling small ants as they move about. They usually do not accept food until the fourth day although a few fed on the third day after hatching. However, the first feeding usually does not occur until 4 to 6 days have elapsed after hatching and lasts for an average time of eight minutes, when the larvae were fully distended and darker in color, no longer accept food and remain quiet until the ninth day when the first molt occurs.

The second larvae measure 4.6 mm. in length. They are at first of a pale brown color but become darker in the course of a few minutes. Our specimens refused to accept food until the fifth day after the first molt when they commenced their feeding which lasted on an average for 9 minutes. It was observed that while sucking a clear yellowish fluid came out of the anus and dropped down on the jar or the skin of the finger. This serum-like fluid dries up very rapidly and does not possess any urticating properties. The second molt occurs ten days after the completion of this single meal. One larva from this batch was fed only four minutes, and then kept in a separate bottle. When all the others had molted, this particular larva was allowed to complete its meal. It sucked for five minutes more, then it molted on the tenth day after it had completed its meal. It thus required the same period of time after the completion of feeding to prepare for molting as did the others that completed their feeding in a single meal. It seems then that it is absolutely necessary to have a full meal and get well distended in order to initiate the changes that culminate in the molt.

The third larvae (fig. 9) measure 6.2 mm. in length. They accept food after four days and their feeding requires 10 to 11 minutes. The third molt occurs 15 days after this single meal. Again one larva from this batch was fed for five minutes only, when it was placed in a separate bottle and two days later it was allowed to suck blood for five minutes more, thus completing its feeding. Its molting occurred two days later than that of the others.

The fourth larvae (fig. 10) measure 9 mm. in length. The first and only meal took place on the eighth day after molting. The fourth molt occurred 26 days after feeding when the nymphae emerged.

The nymphae (fig. 11) measure 17 mm. in length. Their first meal was taken 18 days after the molt. The reduviid at this stage feeds repeatedly and meals were taken 15, 28, 34, and 45 days after molting. Their subsequent feedings were very brief and were made at intervals varying from 10 to 30 days, some taking in all as many as nine meals. An average of three months after the last larvae molted into nymphae, these molted into imagos. Eleven larvae which were carried through the entire cycle furnished nine males and two females. Fifteen days

after their appearance, the imagos accepted food and 20 days later, one of the adult females laid three fertile eggs. Copulation was not observed.

Under the somewhat artificial conditions furnished it in the course of this study, *Rhodnius prolixus* took an average of 200 days to grow from egg to adult, considering the female imago as adult when it was capable of laying eggs. Brumpt reports that in his laboratory at a temperature of 25 C., the entire cycle took only five months, while our insects required an average of seven months to complete the cycle from egg to egg. Considering that under natural conditions, the insect does not procure food so easily and the temperature might be very variable in different localities, it is probable that a much longer period is frequently required to complete the life cycle than that noted under laboratory conditions. The adults reared in the laboratory are smaller than some of the specimens brought from the infested houses. The laboratory females became a little larger after they had laid eggs, but never attained the size of those hatched and reared under normal conditions.

Females are more active, and accept food more frequently than the males. They seem also to be very resistant to adverse conditions. I kept one young female imago for approximately seven months without any food. During all that time it remained motionless in the same place, and was so thin and flattened against the surface of the strip of paper where it rested, that it appeared to be dead, except when touched with a dissecting needle when it showed slight movements of the antennae. This specimen had just one full meal after hatching from the nymphal stage, and died seven months after the meal. During all this time, no eggs were laid and none were found at postmortem.

Larvae in different stages of development were forced to fast for weeks, showing very variable resistance to complete starvation. One "third" larva lasted for five months without taking any food. It was also found that starved larvae survived longer if kept in dark, damp places. Starving larvae and imagos were kept together with larvae and nymphae full of blood and watched during long hours, day and night, but no instance of cannibalistic tendencies were observed as described by Hoffman (1923) for the Cuban reduviid, *Triatoma flavida* Neiva.

Newly hatched larvae were starved for two weeks and then put together with distended larvae and nymphae. In some instances it was observed that the starved newly hatched larvae fed upon the fresh fluid feces of older larvae and nymphae. The feces of these infected Rhodnius contained slender trypanosomes and numerous round aflagellate, leptomonad and crithidial forms. Coprophagism was observed only in newly hatched larvae and only on two occasions. Unfortunately these larvae were not isolated to see whether infection resulted. Drops of water were put in the jar where starving larvae of all ages were kept, but none of them sucked from them.

Whether coprophagism occurs under natural conditions I do not know, but it seems probable. This would then account for the large percentage of *Rhodnius prolixus* infected with *T. cruzi* in the state of Trujillo, Venezuela, and it would then be unnecessary to assume that all the infected Rhodnius have acquired the infection from either man or other animals. It is, however, quite possible that some of the numerous species of mammals living in the infested houses may serve as reservoirs or carriers of *T. cruzi,* from which Rhodnius acquires its infection.

NATURAL INFECTION OF *Rhodnius prolixus* WITH *Trypanosoma cruzi*

Rhodnius in different stages of development were obtained from the towns of Carvajal and Pie-de-Sabana, and examined for *T. cruzi.* Of these specimens I obtained only 6 first larvae, which can be explained by the difficulty of finding them and the short period before they molt into the subsequent stage.

Insects were dissected by cutting the margins of the abdomen with fine scissors, the ventral flap turned up, the entire contents removed and put in salt solution and the intestines teased with the aid of dissecting needles. Mobile forms are easily recognized in fresh preparations. Films were made from specimens which did not show mobile forms, which after fixation were stained with Giemsa, and examined with high power immersion objective. The findings are reported in the following table.

Table Showing the Percentage of Rhodnius Prolixus Naturally Infected with T. Cruzi

Stage of Development	Number Examined	Number Infected	Percentage of Infection
First larvae	6	0	0
Second larvae	18	2	11
Third larvae	32	7	21
Fourth larvae	84	39	46
Nymphae	146	92	63
Adult males	128	84	65
Adult females	139	122	87
Totals	553	346	62
Average percentage of infected adults, male and female			74

SUMMARY

Rhodnius prolixus is strictly domiciliary in habit and at all the stages of its development depends on blood for food. It possesses marked resistance to adverse conditions and can live without food for a period of several months.

Molting occurs after a definite number of days following the completion of feeding. It seems that it is absolutely necessary for this

species to become well distended with a full meal in order to initiate the changes that culminate in the molt.

Under laboratory conditions and at temperatures ranging from 27 C. to 32 C., *Rhodnius prolixus* took an average of seven months to develop from egg to adult.

The insects were fed upon our blood, and the bite was always almost painless. Slight induration was occasionally observed at the site of the bite but no ill effects were experienced.

Numerous starved larvae and adults were kept together with fully distended individuals but in no instance were cannibalistic habits observed.

Newly hatched larvae kept without food were put together with well-fed nymphae and in two instances were seen to feed on the liquid feces which had been recently passed by the fully fed insects. Microscopical examination of the fluid feces showed infective forms of *Trypanosoma cruzi*. Newly hatched larvae may get infected by feeding upon feces from other infected Rhodnius.

Out of 553 specimens examined, 62 per cent were found to harbor different stages of *Trypanosoma cruzi*. The percentage of infected individuals increases as the stages of development are more advanced.

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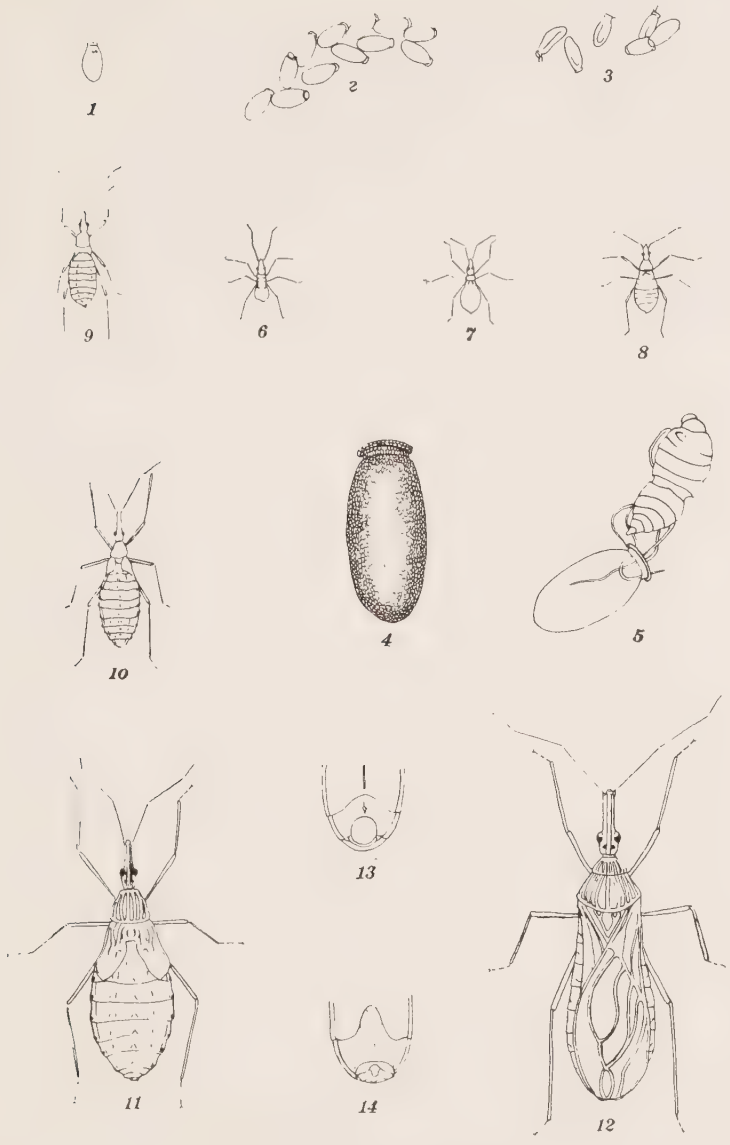

PLATE VII

EXPLANATION OF PLATE VII

All figures concern *Rhodnius prolixus*

Fig. 1.—Egg, × 2.
Fig. 2.—Row of alternating eggs, × 2.
Fig. 3.—Empty shells, × 2.
Fig. 4.—Egg shell showing the granular appearance of its surface, × 12.
Fig. 5.—Larva emerging from the egg, × 8.
Fig. 6.—First larva, × 2.
Fig. 7.—First larva after feeding, × 2.
Fig. 8.—Second larva, × 2.
Fig. 9.—Third larva, × 2.
Fig. 10.—Fourth larva, × 2.
Fig. 11.—Nympha, × 2.
Fig. 12.—Adult female, × 2.
Fig. 13.—Ventral view of posterior extremity of adult male, × 2.
Fig. 14.—Ventral view of posterior extremity of adult female, × 2.

THE NUCLEAR STRUCTURE OF *DIENTAMOEBA FRAGILIS* *

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The writer has recently had the opportunity of studying *Dientamoeba fragilis* in preparations from the feces and in preparations from cultures upon the Boeck-Drbohlav Locke-egg-serum medium. The material upon which this study of the nuclear structure is based consisted of stained specimens from the feces containing the organism prepared by Dr. F. G. Haughwout, of Manila, and kindly loaned the writer by Major George R. Callender, Medical Corps, U. S. Army, Curator, Army Medical Museum, and of stained preparations from cultures furnished by Captain J. H. St. John, Med. Corps, U. S. Army, who has been successful in cultivating this parasite upon the medium above mentioned. It may here be stated that the morphology of the amoebae in cultures and in the feces, including the nuclear structure, was found to be practically identical, although it was noted that in the cultures the binucleate forms were less numerous than in the feces. The material examined was fixed in Schaudinn's solution and stained with hematoxylin.

Dientamoeba fragilis Jepps and Dobell, 1918, is a comparatively small amoeba parasitic in the intestine of man, and characterized by the presence of two nuclei in the majority of the organisms observed in the feces. Uninucleate amoebae of this species always occur along with the binucleate amoebae but Dobell [1] states that in three different cases observed by him about 80 per cent of the amoebae were binucleate. In the stained material from the feces examined in this study slightly over 80 per cent of the amoebae were found to be binucleate but in the material from the cultures not more than 67 per cent of the amoebae were binucleate.

The general morphology of *Dientamoeba fragilis* has been well described by Jepps and Dobell (1918), Dobell (1919), and Dobell and O'Connor (1921), and the only object of this study is to place upon record certain observations regarding the morphology of the nucleus of this species that may prove to be of service in its differentiation and that add to the descriptions of the nucleus by others. The nuclei in the uninucleate and binucleate amoebae are usually of the same size but in the binucleate forms one of the two nuclei may be a little larger than the other. In the binucleate amoebae the structure of the two

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nuclei is, broadly speaking, identical, but in many of these amoebae it was noted that differences occurred in the amount of chromatin in the karyosomes of the two nuclei and in its arrangement, while in the uninucleate amoebae the karyosome of the nucleus in some specimens appeared to contain a larger amount of chromatin, in smaller granules, than the karyosomes noted in the binucleate amoebae. In all of the amoebae observed the nuclei were circular or slightly oval in shape, the vast majority being perfectly round, and in the binucleate organisms the two nuclei were generally at some distance from one another, although numerous amoebae were observed in which the nuclei were in apposition.

The nuclear membrane in this species is exceedingly delicate and often invisible even in well stained preparations, the karyosome appearing to lie within a vacuole in the cytoplasm of the amoeba. When visible the nuclear membrane is poorly stained and so delicate as to require the most careful focussing in order to distinguish it. It is much more delicate than the nuclear membrane of *Endamoeba histolytica,* which, as is well known, has the most delicate nuclear membrane of any of the parasitic amoebae of man with the exception of *Dientamoeba fragilis.*

The writer has been unable to satisfy himself that the nuclear membrane contains any chromatin granules upon its inner surface, although Dobell (1919) states that "as a rule, excessively minute granules can be resolved on or in the nuclear membrane." The writer has observed very minute grains of chromatin staining material on or in the membrane when degeneration was apparently occurring, as evidenced by the irregular staining of the nuclear membrane but has been unable to demonstrate the occurrence of such chromatin grains in organisms in which the membrane stained uniformly throughout. In the vast majority of the amoebae, both from the feces and from cultures, the nuclear membrane was invisible even in preparations containing individuals showing a dimly stained nuclear membrane, so that, as far as diagnosis is concerned, the apparent absence of a definite nuclear membrane is a distinguishing characteristic of the nucleus of *Dientamoeba fragilis* in the majority of specimens encountered in routine examinations. As stated, in most of the amoebae the karyosome appeared to lie within a clear vacuole in the cytoplasm of the amoeba, an appearance quite unlike that observed in any other parasitic amoeba of men.

The structure of the karyosome varies considerably in different amoebae, whether due to cyclical changes occurring in the nucleus during reproduction and development or to degeneration, it is impossible, at present, to state. The structure also varies greatly, as observed in stained preparations, with the degree of differentiation of the stain, and, unless the preparations are well differentiated, the typical appearance of the karyosome is entirely lost, the latter appearing as a solid circular mass of chromatin, stained intensely black, without any definite structure.

The usual description of the karyosome, as given by most writers, states that it is composed of granules of chromatin, loosely arranged, imbedded in plastin, and situated at the center of the nucleus. A careful study of the karyosome of this species in the material mentioned has demonstrated that there is considerable variation in its structure and the illustrations show most of the commonly observed variations. It will be noted that the description of the karyosome as a uniform collection of chromatin granules imbedded in plastin is not correct, in most instances, as there is a great variation in the size of the chromatin granules, in their arrangement and location, and a remarkable agreement in the number of granules that are usually observed within the karyosome. Owing to the relatively minute size of the chromatin granules in the karyosome it is essential that the highest magnification possible with the microscope be used in order to demonstrate much of the data here presented. As usually observed in well stained and differentiated preparations, the karyosome is composed of a few large, deeply stained, distinct granules, imbedded in a less deeply stained material, generally interpreted as plastin. The granules may vary in size but are usually quite large and it is remarkable how frequently they appear to be of the same size, especially in the binucleate amoebae.

The number of granules varies but in the majority of both uninucleate and binucleate amoebae the karyosome consists of four large, deeply stained chromatin granules, circular in shape, imbedded in a circular mass of poorly stained material. Amoebae are frequently observed in which five, six, or even more granules can be counted in the karyosome, and still more rarely, organisms in which the karyosome appears to consist of numerous very minute chromatin granules, but the number usually present is four, and these granules are generally of similar size and stain intensely black with hematoxylin stains.

The arrangement of the chromatin granules in the karyosome varies, the most frequent arrangement of the four granules being in the form of a tetracoccus. As the nuclear membrane is generally invisible, the karyosome apparently lying in a vacuole in the cytoplasm of the amoeba, this arrangement of the chromatin makes the karyosome resemble a tetracoccus lying within a digestive vacuole. Sometimes the granules are arranged in the form of a circle or irregularly, as shown in the illustrations. If numerous small granules are present they are arranged in a loose mass, some of them staining more intensely than others. The arrangement of the chromatin granules in the binucleate amoebae frequently varies in the two nuclei. In the karyosome of one nucleus there may be four large granules arranged in the form of a tetracoccus, while in that of the other nucleus there may be a larger number of granules arranged in a circle, but usually the two nuclei are identical as regards the structure of the karyosome.

While the karyosome is usually situated in the center of the nucleus, not infrequently it may be displaced to one side, some of the granules of which it is composed being in contact with the nuclear membrane, or the karyosome may be broken up and the granules distributed throughout the nucleus, often in contact with the nuclear membrane. Whether such organisms are undergoing degeneration or whether these changes are reproductive in character is undetermined. In the vast majority of the amoebae of this species there is a well defined clear area between the karyosome and the nuclear membrane in which no chromatin granules are visible or traces of a linin net-work. Although the preparations that the writer studied were cell stained and the highest magnifications were used in the examinations, the linin threads described by Dobell and others as extending from the karyosome to the nuclear membrane have not been observed, although in a few organisms there were noted faint shadows indicating that such linin threads might be present but, owing to their delicacy, were decolorized during the differentiating process.

Dividing forms were not observed in any of the preparations with one exception. In one preparation from the feces a single uninucleate amoeba was noted in which the nucleus was much elongated and the four granules in the karyosome were arranged in a straight line stretching across the long diameter of the nucleus. It is believed that this was a dividing form but it was the only one observed in the study of hundreds of amoebae of this species. The preparations from the cultures did not throw any light upon the method of reproduction of this interesting species of amoeba. Although searched for most carefully in preparations from both the feces and cultures, no cysts of *Dientamoeba fragilis* were observed. Cysts of this species have been described by Kofoid but the writer was not able to demonstrate cysts in the feces or in the material from cultures that he has examined.

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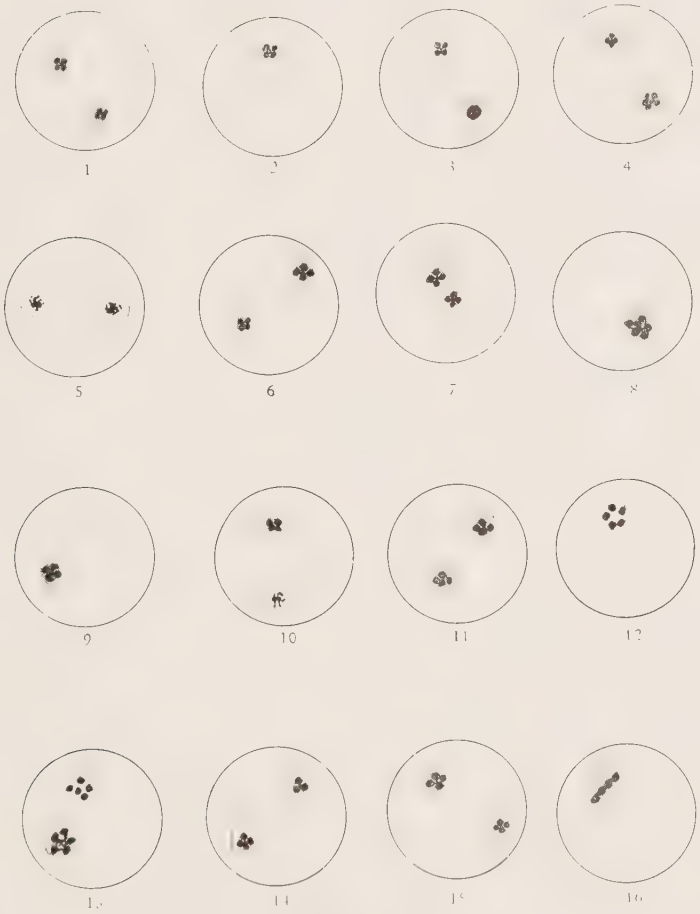

PLATE VIII

Explanation of Plate on Next Page

Explanation of Plate VIII

The figures are free-hand drawings, the body of the amoeba being represented by the large circle. The drawings of the nuclei were made from actual specimens of the amoeba from the feces and cultures. Magnification approximately $\times 2200$.

1. Binucleate amoeba, with karyosomes of same size and each containing four chromatin granules imbedded in plastin. Note the very delicate, poorly stained nuclear membrane. In one of the nuclei the karyosome is excentric in situation.

2. Uninucleate amoeba, with typical karyosome.

3. Binulceate amoeba, in which one karyosome is apparently composed of a solid mass of chromatin while the other contains four distinct granules of chromatin.

4. Binucleate amoeba in which the karyosome of one nucleus contains four chromatin granules while the karyosome of the second nucleus contains five chromatin granules and is larger.

5. Binucleate amoeba in which both karyosomes are composed of fine granules of chromatin.

6. Binucleate amoeba in which the karyosome of one nucleus is much larger than the other but both are composed of four chromatin granules.

7. Binucleate amoeba, with nuclei in contact.

8. Uninucleate amoeba with karyosome containing six chromatin granules, filling almost entire nucleus.

9. Uninucleate amoeba with karyosome eccentrically situated and in contact with the nuclear membrane.

10. Binucleate amoeba in which the karyosome of one nucleus contains four chromatin granules situated to one side of the nucleus in contact with the nuclear membrane, while that of the second nucleus is composed of fine granules of chromatin.

11. Binucleate amoeba with typical karyosomes.

12. Uninucleate amoeba in which the karyosome, composed of five distinct chromatin granules, is broken up and the granules distributed in the nucleus in contact with the nuclear membrane.

13. Binucleate amoeba, in which the karyosome of one nucleus is composed of five granules distributed in the nucleus, while that of the second nucleus is composed of five chromatin granules apparently connected.

14 and 15. Binucleate amoebae, illustrating differences in the structure of the karyosome.

16. Dividing amoeba, in which the chromatin granules of the karyosome are arranged in a line within the elongated, oval-shaped nucleus.

ARE *ASCARIS LUMBRICOIDES* AND *ASCARIS SUILLA* IDENTICAL?*

FRED C. CALDWELL
ELFREDA L. CALDWELL

The case for the positive side of the question proposed is started by Cram (1926) as follows:

> "It was long debated whether the ascarid of man and that of the pig are identical. Ransom (1922) states that they are certainly of the same species, that there is no difference morphologically and that, according to Barker, they cannot be distinguished by the complement fixation test. Schwartz (1920) also concludes from precipitin tests on the two forms, that they are biochemically indistinguishable. Barker (1922) finds the chromosomes identical."

Throughout her paper Cram assumes that the ascarids of man and the pig are identical and states specifically that "the presence of *Ascaris lumbricoides* means that the person infested has swallowed the alvine discharges from human or *swine* intestines." The *Journal of the American Association* comments editorially (1926) that "there is no longer any question that the ascarid of the pig and man are identical." Bakker (1921), who made morphological, toxicological, and serological studies, concludes that there is no difference between the two ascarids.

In opposition to this conclusion are the findings of Koino (1922). After swallowing 2,000 mature eggs of the human Ascaris, he developed pronounced pulmonary symptoms with blood sputum, which contained larvae, and later recovered 667 worms. From another subject, who swallowed 500 ova of *Ascaris suilla* and who developed but mild symptoms, no larvae or worms were recovered. The work of Payne, Ackert, and Hartman (1925) supports the finding of Koino that fertile ova of the pig ascarid do not develop to maturity in man. Furthermore, they found that embryonated ova of the Ascaris of man, when fed to pigs, failed to grow to maturity and produce ova. They cite the marked difference in incidence of Ascaris in man and pigs in Trinidad, under conditions conducive to the spread of ascariasis, as an indication that the pig and man are not reciprocal hosts for these parasites. The incidence ranged from 20 to 70 per cent in man and from 3.5 to 10.8 per cent in pigs. They conclude that "there is a physiological difference between the two ascarids of a degree sufficient to make it impossible for the eggs of the human Ascaris to produce mature ascarids in pigs or for the eggs of the pig Ascaris to produce mature ascarids in man."

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* Study made at the Hookworm Research Unit of the International Health Board of the Rockefeller Foundation, Andalusia, Alabama.

To investigate further the epidemiological aspect of this problem a survey was made by the writers in Covington County, in the sandy coastal plain of Alabama, where the annual mean temperature is about 66 F. and the annual mean precipitation is about 53. In the examination of 444 rural white children in the lower coastal plain in 1923 Augustine found 96.4 per cent positive for hookworm, while Ascaris was found in but 2 per cent of these cases (personal communication in 1925). Of 220 urban children examined, 40.1 per cent were infested with hookworm; none with Ascaris. Of the specimens of human feces examined in 1925 in the laboratories of the Alabama State Board of Health (personal communication from Dr. L. C. Havens) 34 per cent were found positive for hookworm. In marked contrast to this is the Ascaris incidence of only 1.5 per cent. In view of this general situation the Ascaris incidence in pigs should have peculiar significance. It was therefore proposed (1) to examine the feces of a sufficient number of pigs to determine the extent of ascariasis in swine; (2) to examine the feces of the members of the families owning the pigs to establish their incidence of infestation with Ascaris; in order (3) to determine the possibility of cross infestation between humans and pigs.

We are indebted to Dr. N. R. Stoll for valuable suggestions, to Dr. L. C. Havens and Dr. D. L. Augustine for the results of examinations of specimens of feces made in their work, to Drs. J. A. Kerr and E. R. Rickard for the use of data secured in a field survey in Tennessee, and to Dr. J. A. Kerr for like data obtained in the course of a survey of intestinal parasites in school children which is in progress in Florida.

METHODS AND RESULTS

The specimens of feces from the pigs were collected personally by the writers to ensure that no duplications would occur. Notes were made of the actual or apparent age of each pig, of its physical condition, and of its general surroundings. In some instances the pigs ranged freely throughout the year; in the majority of instances they were confined in pens from the planting season to the time of harvest, after which they were turned out in the fields; in a few instances the pigs had been confined in pens practically from birth. The size and sanitary conditions of these pens varied considerably. At the time of the survey the majority of the pigs in the region had been turned out to range, so that the amount of material available was limited and difficult to locate.

An attempt was made to collect specimens of feces from all members of families owning pigs found infested with Ascaris, and, on the whole, we secured excellent co-operation.

Both pig and human specimens were examined for ova of Ascaris by the brine flotation method. Because of the great amount of vegetable

fiber present in the stools of pigs, the specimens found negative by brine flotation were also examined by either the centrifuge method or antiformin-sugar technic (Caldwell, 1926).

A total of 247 pigs were examined. These were owned by 25 different families. Of these pigs 115, or 46.5 per cent were infested with Ascaris. The swine infested were owned by 16 families comprising 78 individuals, from all but five of whom we secured samples of stools. No member of any of these families was found infested with ascarids, though 51 or 65.4 per cent had hookworms. Also during the course of this survey 396 school children in Covington and two neighboring counties in the lower coastal plain of Alabama were examined for intestinal parasites. Of all these children only one had ascarids, whereas 310 or 82.4 per cent had hookworms.

DISCUSSION

Cram (1926) presents the following facts with regard to the distribution of ascariasis and cites authorities for her statements: Ascaris infestation is more prevalent (1) among children than adults; (2) in rural as opposed to urban communities; (3) in tropical and sub-tropical as compared with temperature climates; (4) in regions of heavy rainfall in contrast with dry areas; (5) in places where sanitation and economic conditions are bad. She further emphasizes the several ways in which humans may become infested with Ascaris, namely: "(a) the use of excreta of human or swine origin for fertilizers; (b) the contamination of vegetables as a result of (a) or otherwise; (c) the contamination of water; (d) the contamination of the hands and feet of persons; and (e) the spread by insects and other animals."

In view of the above statements, if the ascarids of the pig and human are identical, it would be most difficult to explain the findings of our survey. The study was conducted in a region of sub-tropical climate with abundant rainfall, in a rural community lacking sanitation and with poor economic conditions, with a fair number of young children, presumably susceptible to Ascaris infestation. All the conditions cited by Cram are apparently favorable for the dissemination of Ascaris. In this connection the high incidence of hookworm is significant in that it indicates that the given conditions are instrumental in spreading filth-borne diseases. The specific routes which Cram gives for the infestation of humans with Ascaris were wholly or partially operative in all the homes investigated in this survey, so that, if it is possible to infest man with pig Ascaris, such infestation should have been in evidence here. The pens were but a few rods from the houses, which were unscreened and swarmed with flies. Chickens, dogs, and cats were in evidence. The

children ran barefoot, as did some of their parents, and clambered in and out of the pens. No sanitation was present. Many of the young pigs infested with Ascaris ran freely about the yards and were petted and handled by their owners. The water was drawn from shallow, open wells. The majority of the swine ranged in the fields and gardens during at least a portion of the year. Yet Ascaris in humans in the lower coastal plain of Alabama is practically non-existent, whereas almost 50 per cent of the swine are infested.

The general situation revealed by the survey is provocative of speculation and suggests several interesting lines of investigation. In a region where the incidence of pig Ascaris is high, and where the infestation of the human population with hookworm is heavy, why is the human Ascaris rare? In this connection the results of a survey recently concluded by Kerr and Rickard (1926) in Tennessee are of great interest. In Tennessee a high incidence of intestinal parasites was found only in certain sections of the eastern part of the state. In the Unaka Mountain district, for example, the percentage of children infested of those examined was as follows: Ascaris, 58 per cent; Trichuris, 59 per cent; hookworm, 69 per cent. As is evident from these figures a high incidence of Ascaris in humans is associated with high incidence of Trichuris and hookworm. In the plateau of west Tennessee there was found no infestation with hookworm or Ascaris, and but 3 per cent with Trichuris.

A recent field survey in Florida reveals a situation somewhat similar to that in South Alabama, namely, a high incidence of hookworm with practically no Ascaris or Trichuris. Of 1,956 specimens examined (courtesy of Dr. J. A. Kerr), 1,178 were positive for hookworm, but only 23 for Ascaris, and 44 for Trichuris.

Economic, social, and sanitary conditions in the rural districts of all these areas are similar. It would seem, therefore, that in the sandy, coastal plain there must be present other factors which are unfavorable for the development of human Ascaris. One of these factors may be the nature of the top soil. The role which variations in soils may play in the distribution of ascariasis among humans and pigs is now under investigation by us.

CONCLUSION

In an area in which the incidence of Ascaris infestation in pigs is 46.5 per cent and in which all factors would seem to be favorable for cross infestation between pig and man, the incidence of human Ascaris is less than 1 per cent.

This survey adds epidemiological evidence to the experimental evidence of Koino, Payne, and others, that the pig and human Ascaris are not identical.

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MORPHOLOGICAL DIFFERENCES BETWEEN NECATOR AND ANCYLOSTOMA LARVAE*

RUTH M. SVENSSON AND JOHN F. KESSEL

During 1924 in Peking and in Soochow, while carrying on some experimental work in connection with the investigation of the China Hookworm Commission, and in 1925, while working in the Parasitology Laboratory of the Peking Union Medical College, we have had an opportunity to examine large numbers of infective larvae of both species of hookworm which infect man, *Ancylostoma duodenale* and *Necator americanus,* as well as those of the dog species, *Ancylostoma caninum.* At the suggestion of Drs. W. W. Cort and N. R. Stoll of the China Hookworm Commission, a careful study was undertaken to ascertain whether some clearly definable morphological distinctions exist between these larvae, which would make it possible to determine the species of hookworm present in fecal cultures and in polluted soil. This investigation was considered to be the more valuable, since in surveys which precede campaigns for the control of hookworm disease it is of great importance to obtain information concerning the type and degree of soil infestation as well as of infection existing in the population.

Darling's (1922) method of choosing a representative and sufficiently large group of the population, to which he administered an anthelmintic, and then counting and identifying the worms expelled during seventy-two hours following the treatment, undoubtedly gives the most exact information regarding the species present. Serious difficulties, however, are encountered in the application of this method because of the great expense involved, the skilled work required, and the difficulty in securing cooperation from the population. It is, therefore, important that methods be found which give the same information as Darling's method but which are more easily applicable as far as expense and cooperation from the population are concerned.

The egg-count method devised by Stoll (1923) enables the worker in a preliminary survey to estimate fairly accurately the degree of infection in any group of people. It can be handled easily even by purely mechanically trained assistants, is not expensive, and for a rough estimate does not require more cooperation from the population than the delivery of a single fecal sample per person. However, it has the disadvantage, compared with Darling's method, that no information can be gained regarding the species of hookworm involved.

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* Contribution no. 75 from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.

The Baermann apparatus for isolating hookworm larvae from the soil (Baermann 1917) has made it possible to localize the foci and to determine the degree of soil pollution. Here again, however, the information regarding the species of hookworm larvae present in the soil has not thus far been obtainable, since the different hookworm species have, prior to our investigation, never been differentiated in the larval stage.

MATERIAL AND METHODS

The larvae used for our study were obtained from the following sources:

1. Cultures from three human cases of known experimental infection with *Necator americanus*.

2. A case in the Peking Union Medical College Hospital where examination for the adult worms expelled after treatment showed the presence of only *Ancylostoma duodenale*.

3. Several cases in the Peking Union Medical College Hospital where examination of the worms expelled after treatment showed the presence of both *A. duodenale* and of *N. americanus*.

4. Dogs infected with *Ancylostoma caninum*.

After the differentiation had been made from the pure cultures, numerous larvae, cultured from human cases where no identification of the adult worms had been made, were used for further study. Larvae isolated from soil in Soochow and in Canton or cultured from the contents of the "kangs" used for storage of human feces, have also been studied.

In young infective hookworm larvae (filariform stage) very few details of the internal structure can be observed, owing to the fact that large numbers of food granules have been stored up around the cells of the intestinal wall during the earlier rhabditiform stage. It is necessary, therefore, to reduce these granules before a careful morphological study can be attempted. This can be accomplished either by leaving the larvae in soil cultures four or five weeks or by rapidly aging them artificially through increased activity. The latter method which is the more convenient one has been generally utilized in this investigation. It is based on the demonstration of the vertical migration of hookworm larvae by Payne (1923). Her method was to bury larvae near the bottom of a glass tube under a column of moist sand; the larvae then migrated to the surface where they could be collected.

To save time and work a modification of this method has here been employed. A glass or tin tube 4.5 cm. in diameter and 12 cm. high was placed in a Petri dish and filled to within 4 cm. of the top with sand which had previously been sterilized by heating. The fecal sample containing the hookworm ova was then placed on the sand and

the tube filled with sterilized soil. The culture was moistened and kept at room temperature for five days to give the larvae time to hatch and to reach the infective (filariform) stage. Another Petri dish was placed over the upper end of the tube to prevent desiccation and to allow for inversion of the culture if required. After two or three days the larvae had migrated to the surface. By this time the food granules were reduced to such an extent that a clear view of the internal organs of the larvae was afforded. When a further reduction of the granules was desired, it was obtained either by placing another tube filled with sand on the top of the first one or by inverting the tube again, thus causing the larvae to migrate through it once more.

If a large number of larvae was desired, the entire contents of the tube were isolated in the Baermann apparatus, but if only a few larvae were needed for identification a simpler and quicker method was employed. One or two grams of sand were taken from the surface of the culture and tied into a small linen bag. This was placed on a large slide and sufficient warm water was poured over it with a pipette to moisten the sample and still leave a medium-sized drop on the slide. In one or two minutes the larvae would pass through the meshes in the cloth and swim out into the water-drop on the slide. If the slide were gently warmed from below, the larvae left the bag more readily. The bag was then removed, a coverslip was placed on the drop of water, and the larvae were ready for study. If too much sand had come through onto the slide, a cleaner preparation was made by placing another coverslip with its edge touching the first one. Most of the sand remained under the first coverslip while water and several larvae were drawn under the second one, forming a very clean preparation.

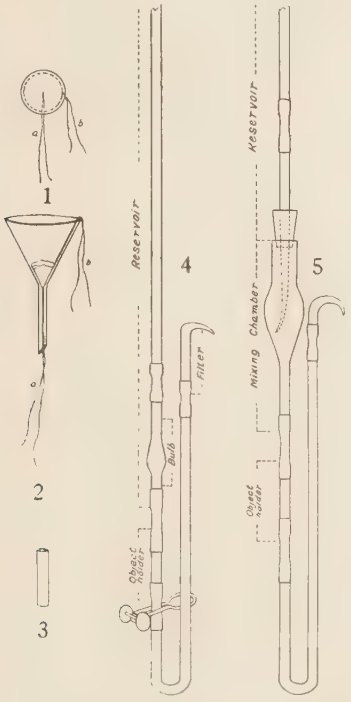
If the larvae were too active, they were temporarily quieted down by placing the slide on ice for a minute. They could be inactivated for a longer time if the preparation was sealed with a heated mixture of equal parts of resin and paraffin and left in strong light for about an hour, preferably on the microscope stage in full illumination from a microscope lamp.

In staining fixed larvae, the technic of Cobb (1890) and Magath (1916) was followed with modifications as indicated below.

1. Larvae with their food granules suitably reduced, were allowed to sink to the bottom of a test tube and the supernatant water was then pipetted off. The larvae, suspended in as small an amount of fluid as possible, were then killed in 50% alcohol at 70° C.

2. A circular piece of linen or silk of fine texture (Textfig. 1), 2 cm. in diameter, was equipped with (*a*) one thread through the center and (*b*) another thread stitched in and out around the edge but with free ends. With the aid of the thread (*a*) the linen piece was inserted into a little glass funnel as in textfigure 2. The larvae suspended in

the killing fluid were strained through the funnel, using a wide-mouthed pipette to collect and transfer them uninjured. When all the larvae had been caught in the cloth, this was tied into a bag by using the thread (*b*) as a draw-string, and was then inserted into the object holder (Textfig. 3), a piece of glass tubing 10 mm. in diameter and 4 cm. long, again using thread (*a*) as a leader. (A fine crochet-hook was found to be very helpful in drawing the thread through the tubing).



3. The object holder was then placed for two hours in a test tube containing the fixing fluid which was prepared as follows:

Acetic acid	)
Alcohol (95%)	) equal parts
Water saturated with corrosive sublimate	)

4. The object holder was then placed in position in the staining apparatus (Textfig. 4). This corresponded closely to the differentiator described by Cobb (1890) but was modified, in that (1) the reservoir was provided with a glass bulb to catch the air bubbles, and in that (2) the filter reached above the object holder to prevent it from running dry. The filter was first filled with 35% alcohol and the clamp screwed tight. Then the object holder, filled with alcohol of the same strength, was attached and a cotton plug inserted. The reservoir was attached

and filled with 35% alcohol containing sufficient tincture of iodine to give it a dark amber color. For twelve hours about 30 cc. of the iodine-alcohol was allowed to pass through the apparatus, the flow being regulated by the clamp.

5. Distilled water was passed through the apparatus for about an hour.

6. The apparatus was filled with Delafield's hematoxylin (1 : 25 dilution) and left for twenty-four hours.

7. Water was then passed through the apparatus for twenty minutes.

8. Acetic acid (5%) was rapidly passed through for five minutes, followed immediately by distilled water.

9. Ammonia (5%) was then passed through for five minutes and this again followed by distilled water.

10. For dehydration the object holder was transferred to Magath's differentiator (Magath, 1916) having the mixing chamber (Textfig. 5). Magath's apparatus differed from the one described above (Fig. 4) in having a wider and longer piece of tubing as a reservoir, enough to hold about 50 cc., and having a mixing chamber into the bottom of which the fluid contained in the reservoir was forced. As the various grades of alcohol were poured into the reservoir in increasing strength (i. e., decreasing specific gravity), a lighter fluid was forced through a heavier fluid, and a thorough mixing took place before the alcohol passed down through the object holder, thus preventing the larvae from being exposed to any sudden changes in osmotic pressure. The reservoir was successively filled with 35%, 50%, 70% and 85% alcohol. When it had nearly emptied itself, 20 cc. of 95% alcohol was added and then absolute alcohol until 50 cc. of the later had been used. When only absolute alcohol remained in the reservoir, a glass tube like the object holder containing CaO and plugged with cottonwool in both ends, was attached to the upper end of the reservoir to avoid the absorption of moisture by the absolute alcohol. The flow was then regulated so as to allow twenty-four hours for the dehydration process.

11. The object holder was removed from the differentiator and placed in a little stender dish containing absolute alcohol. Two more stender dishes were placed in position, one higher and one lower than the dish containing the specimen. The upper dish was filled with a mixture of equal parts of absolute alcohol and synthetic oil of wintergreen (methylsalicylate). A syphon system was arranged in the three dishes with strings serving as wicks. When the highest dish had emptied itself into the one containing the specimens, it was filled with pure oil of wintergreen. In this way the specimens were brought into pure oil of wintergreen in thirty-six hours.

12. The linen bag containing the larvae was then opened and the specimens mounted on a slide in a drop of damar in oil of wintergreen.

MORPHOLOGICAL DIFFERENCES OBSERVED

Close observation revealed the fact that there was a great deal of difference in the shape of the esophagus of the larvae of various species of free-living nematodes. It was also apparent that the spear located anterior to the esophagus differed in shape and structure in different species of larvae. Cobb (1923) figured this structure for *Necator americanus*.

While studying the larvae from the material at our disposal it became evident that these two morphological characters were different in the larvae of *Ancylostoma duodenale* and in those of *Necator americanus*. A description of the appearance of these differences as observed with paired 5X oculars, using 16 mm., 4 mm., and oil immersion objectives (Leitz binocular research microscope) follows.

A. *The spear.*—If the anterior end of the larvae in lateral view is studied under a 16 mm. objective, a dark rod-like structure is seen in the Necator larvae (Fig. 1), while Ancylostoma larvae appear uniformly transparent or show only a light longitudinal stroke. (Fig. 2). Under a 4 mm. objective and still more distinctly under an oil immersion objective this difference can be easily confirmed. In Necator larvae the mouth cavity is filled with a highly refractile hyaline substance, presumably chitin, in the form of a spear with a broad bifurcated anterior end (Figs. 1, 3). At first glance this spear seems solid, but careful observation reveals a narrow groove inside. In Ancylostoma larvae two narrow longitudinal lines are observed separated by a wide space (Fig. 2). The dorsal line is decidedly heavier, carries at its anterior end a barb-like process, and can be followed posteriad into the dorsal wall of the esophageal lumen and anteriad frequently to the very point of the head. The ventral line can sometimes be indistinctly traced to a point where it joins the dorsal line anteriorly and can be seen to continue into the ventral wall of the esophageal lumen, but as a rule it appears to end without continuation either anteriorly or posteriorly. In *A. caninum* the difference in density of the dorsal and ventral lines is less pronounced than in *A. duodenale*.

Cobb (1923) has observed this same structure that is here described in the anterior end of the *Necator* larvae and from certain likenesses with spear-bearing free-living nematodes has concluded that it was probably a protrusile spear. We have been able to confirm this assumption, actually observing the protrusion of an oral spear not only in Necator but also in *A. duodenale* and in *A. caninum* larvae. If the larvae are placed in a drop of chloroform water on a slide and mounted under a coverslip, almost every unsheathed larva will be found just prior to death with the hollow spear more or less protruded. As the larvae keep moving all the time, we have not been able to make any

camera lucida drawings of this stage in the living larvae, but several of the stained larvae have shown the spear protruded (Fig. 8). In a few cases sheathed larvae have been observed to protrude the spear in chloroform water, but this is not the rule. In one case an *A. duodenale* larva was observed to retract a protruded spear.

B. *The esophagus.*—When unstained, living hookworm larvae, in which the internal morphology is not obscured by the stored food granules, are studied under a 16 mm. objective the esophagus and intestine in Necator larvae appear to be separated by an open space distally marked off by a definite transverse line (Fig. 1), while in larvae of both *A. duodenale* and *A. caninum* the intestine is seen to follow as a direct continuation of the esophagus (Fig. 2). A careful study with an oil immersion objective provides the explanation of this optical impression. The part of the alimentary canal following immediately behind the esophagus, and probably corresponding to the cardia and the intestinal valves of adult nematodes (Looss 1905: 89-90) has a very low refractive index and is almost hyaline in appearance in Necator larvae (Fig. 3), whereas this part of the digestive tract in Ancylostoma larvae (Fig. 5) is of approximately the same coarsely granular character as the esophagus proper. Furthermore, the intestinal valves of Necator larvae are bluntly conical and the attachment to the intestine, into which they are usually somewhat invaginated, is in the form of a narrow circular band around the lumen. The invagination causes a transverse folding of the anterior part of the intestinal wall. In high and in low focus this fold appears as a transverse line, which stands out very strikingly under low magnification when the whole thickness of the larva is near focus at the same time, and when the optical impression of the part of the fold behind as well as the part in front of the cone reaches the eye of the observer simultaneously. Between this line and the highly refractile distal part of the esophagus the cardia together with the intestinal valves appear as an open space. In Ancylostoma larvae this structure is cylindrically shaped and broadly attached to the intestine, so that no invagination into the narrow intestinal lumen can take place and thus no impression of a transverse line be formed. These observations on the fresh larvae are fully confirmed by the study of stained specimens (Figs. 6 and 7).

In a few cases, especially in old larvae which had been kept in water for a long time, the Necator type of spear has been observed in larvae which did not show the space between the esophagus and intestine. A careful study with the oil immersion lens has shown these larvae to be in a state of contraction with the valves completely invaginated into the anterior end of the intestine, thus bringing the transverse line marking the anterior end of the intestine (Fig. 4), as described above, and the posterior demarcation of the esophagus proper into one plane, and

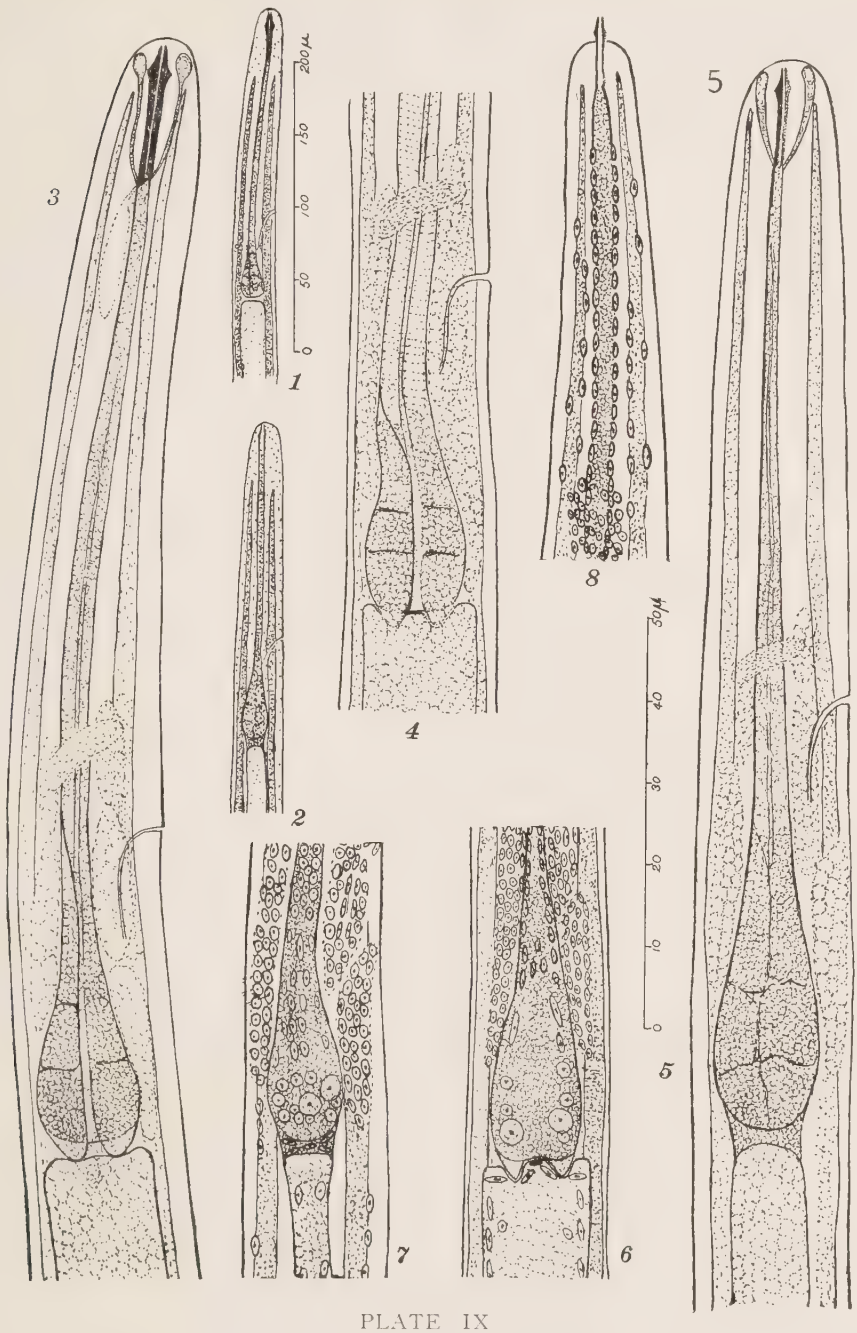


PLATE IX

DESCRIPTION OF PLATE IX

Figures 1-5 are lateral views of unstained free-living forms.

Fig. 1.—Filariform *Necator americanus* larva.

Fig. 2.—Filariform *Ancylostoma duodenale* larvae.

Fig. 3.—Filariform *Necator americanus* larva extended.

Fig. 4.—Filariform *Necator americanus* larva contracted.

Fig. 5.—Filariform *Ancylostoma duodenale* larva.

Figs. 6, 7, 8.—From material stained with Delafield's hematoxylin, nuclei easily distinguishable.

Fig. 6.—Contracted larva of *N. americanus*.

Fig. 7.—Larva of *A. duodenale*.

Fig. 8.—Larva of *A. duodenale* with protruded spear

thus almost entirely eliminating the impression of a space between esophagus and intestine. This is frequently the case with the stained larva (Fig. 6), but with the constancy of the difference in the spears, no real confusion in the distinction of the larvae can be caused by this phenomenon.

The differences in the oral spear and in the union of the esophagus and intestine which have here been found to exist between *Ancylostoma duodenale* larvae on the one hand and *Necator americanus* larvae on the other are equally apparent in comparing the larvae of *A. caninum* with those of Necator. It seems probable, therefore, that the differences pointed out in this paper are of generic as well as of specific value.

We wish to express our appreciation to Drs. W. W. Cort, N. R. Stoll, and E. C. Faust, for assistance in obtaining material and for helpful suggestions during the work.

SUMMARY

1. Infective larvae of *Necator americanus* differ from those of *Ancylostoma duodenale* and *A. caninum* in (a) A protrusile oral spear is present, but different in shape and strength for the two genera. (b) At the point of union between esophagus and intestine, an intermediate transverse space is apparently present in Necator, while in Ancylostoma these regions appear to be in direct contact.

2. A rapid technic for preparing larvae for study is described.

3. A simple and rapid method for isolating a small number of larvae is described.

4. A modification of Cobb's and Magath's technic for staining is given.

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BOOK REVIEWS

A Synopsis of the Families and Genera of Nematoda By H. A. Baylis and R. Daubney. 277 pp. British Museum (Natural History), London.

The recent publication of this synopsis represents the second important contribution of English helminthologists to the confused field of Nematode taxonomy. Yorke and Maplestone confined their survey to the field of parasitic forms found in the Vertebrates, while Baylis and Daubney have included the entire field of Nematoda proper, both free-living and parasitic. For the first time in published form has a complete survey of the entire list of valid genera as of 1923 been arranged into a unified whole and made available for the general use of helminthologists at large. These two books should prove of inestimable value, particularly to the isolated worker.

In attacking so wide a field, the authors have been forced to eliminate all references to other than type species and content themselves with giving diagnoses only down to the genera. A very valuable aid to the general worker is to be found in the list of definitions given in the Introduction. By reference to this list one is able to correlate many seeming discrepancies in the descriptions of several familiar genera, the attempt to reduce to a common nomenclature having involved some apparently striking variations from the accepted statement. The necessity of eliminating all figures is greatly to be regretted but the reason is easy to comprehend; inclusion of such a feature would have rendered the volume unwieldly and perhaps delayed its appearance for a considerable period.

The authors in arranging the genera into larger groups have adopted a much more conservative viewpoint than that advanced by Yorke and Maplestone, particularly in reference to the position of the Heterakidae and Oxyuridae among the parasitic forms. They have also avoided the very radical views advanced by several specialists among students of free-living forms. They admit that the free-living forms are probably ancestral and not specialized but have built their taxonomic schema around the three-lipped forms, the Ascarids, since these are better known to the greater number of workers and also are of possibly greater economic importance according to present ideas. With the exception noted above, the proposed schema not only is simple but also appears as a distinct step towards the all-desired end of a natural classification. In viewing the proposed arrange ment as a unit it is seen that a necessary reduction and simplification of the schemes advanced by specialists in various groups has taken place, splitting of genera and the formation of sub-families, for various forms has been avoided when possible. The authors have themselves been forced, however, to indulge in the current tendency to expansion in that they have abandoned the well known super-family group and raised these units to the importance of orders. The Order Eunematoda has been abolished for the Class Nematoda and the Order Gordiacea has been raised to a par with the classes Nematoda and Acanthocephala. The Class Nematoda of these authors is limited to forms of the Eunematoda of other writers. Such a change may perhaps tend for simplicity but it also adds some confusion to those who have come to regard the super-family as a necessary unit in nematode taxonomy. In raising these groups to the rank of orders several of the familiar groups have been eliminated; the usual eight, Rhabdiasoidea, Oxyuroidea, Ascaroidea, Strongyloidea, Dioctophymoidea, Trichuroidea, Spiruroidea and Filarioidea, being reduced to five by the uniting of the first three into the Ascaroidea, the retention of Strongyloidea and Dioctophymoidea, the questionable substitution of Trichinelloidea for Trichuroidea and the inclusion of the Spiruroidea with the Filaroidea. The inclusion of all of the free-living forms in the Ascaroidea in addition to the parasitic forms usually placed in the Oxyuroidea makes the former a very unwieldly group. As a suggestion the original groups of the Ascaroidea, Oxyuroidea and Rhabdiasoidea might well be retained—with proper spelling—as sub-orders of an order, the name of which should indicate the only outstanding common structural condition of the group, i. e., the possession of a basic three-lipped arrangement. The classification as it stands is very conservative, fairly natural, and above all, very usable and hence indispensible to any worker in the general fields of zoology or parasitology who is called upon to deal with nematodes.

The press work is well done and the print easy to read. For a book so largely dealing with scientific terms there is a surprising lack of typographical errors which shows great care both on the part of the publishers and on that of the authors who are to be congratulated in presenting so difficult a subject in so simple and yet so satisfactory a manner.

MEDICAL REPORT OF THE HAMILTON RICE SEVENTH EXPEDITION TO THE AMAZON, IN CONJUNTION WITH THE DEPARTMENT OF TROPICAL MEDICINE OF HARVARD UNIVERSITY. By R. STRONG, G. SHATTUCK, J. BEQUAERT, and R. WHEELER, 313 pp. 70 pl., 6 text figs. Harvard University Press, Cambridge.

It would be exceedingly difficult within the space at command to do justice to the fine volume in which the work of this important expedition is so well presented. It covers a broad field scientifically from the interesting account of the Amazon forest through medical, parasitological and ethnological data to the technical descriptions of mollusks and insects with which the volume closes. Material of deep interest to the parasitologist is abundant. One section on the Spironemata, others on Leishmaniasis, Trypanosomiasis and splenomegaly, while preponderatingly clinical, contain much on the organisms themselves including admirable photographs and colored plates. Some other parasitic infections of animals and the pathological conditions produced by Arthoroda are also treated in the first (general) part of the report. Part II is an exhaustive study of Medical and Economic Entomology covering not only the region traversed by the expedition but also including comparative data from the travels of one author (Bequaert) in Africa and other tropical regions. Part III contains among other items an article on a new mammalian cestode and a dipterous parasite of a snail from Brazil. It is not possible here to give any adequate idea of the mass of valuable material which the book contains. In appearance as well as in content it reflects great credit both on the institution and on the members of the expedition.

DIE TIERISCHEN PARASITEN DES MENSCHEN. DIE VON IHNEN HERVORGERUFENEN ERKRAKUNGEN UND THRE HEILUNG. By M. BRAUN UND O. SEIFERT. Zweiter Teil. 574 pp., 21 figs. Curt Kabitzsch, Leipzig.

The recent (sixth) edition of the first part of this well known and highly valued work was reviewed last year (The JOURNAL, 12: 57, Sept., 1925). The second part dealing with clinical and therapeutic features of human parasites was last revised in 1920 (see The JOURNAL, 7: 103, Dec., 1920). The present edition is considerably larger than the previous and has been thoroughly worked over. The most extensive additions have been made in the chapters on Haemosporidia and on other groups of Protozoa though omissions and additions have been noted in almost every section. On the other hand this edition still shows some of the minor defects which were noticeable in the second edition. The author is not alive to the value of foreign literature as he confines his references almost exclusively to German publications. Some very important and extensive contributions in other languages are not even mentioned. The arrangement of the material is confused and one finds extensive data concerning Nematoda under Cestoda for example. In these respects this part does not reach the high standard maintained by the first part, the original text on human parasites by Professor Max Braun. However, it should be said that the second part contains the only clinical study of the subject available. It gives a full presentation of the German literature and abundant references to the sources of information and will be of value to all workers in this field.

PRÉCIS DE PATHOLOGIE MEDICALE. MALADIES INFECTIEUSES. By F. BEZANÇON et A. PHILIBERT. 2 vols., 539 pp., 75 figs., and 646 pp., 91 figs. Masson et Cie., Paris.

A series of distinguished French medical teachers have been preparing a Précis of Medical Pathology of which the first and second volumes are at hand. The work is an attractive and concise presentation of infective diseases, classified

according to etiology. Bacterial diseases comprise the major part of the treatise but the second volume contains sections on the diseases due to species of Treponema and those caused by animal parasites. The material is necessarily limited in extent but well selected, the presentation clear and the form attractive. One might wonder why in the section of diseases due to animal parasites the authors confined their attention exclusively to protozoan parasites. Most parasitologists think it is difficult to separate protozoal diseases entirely from those caused by metazoan parasites on any good general grounds.

COPROLOGIE MICROSCOPIQUE. By LANGERON et RONDEAU DU NOYER. 132 pp., 129 figs. Masson et Cie., Paris.

The book by Langeron and Noyer is a convenient laboratory manual in an entirely new field. It is cast along preeminently practical lines and will fill a gap in the literature of parasitology. The book opens with a section on the technique of fecal examinations, takes up next the study of normal and abnormal elements in the stool. Then follows the account of parasites and their products, first Protozoa, then Helminthes, and later Arthropoda and fungi. The index is unusually good. The figures are numerous and reproduce very faithfully the true appearance of the objects as they turn up under the microscope in fecal examinations. The little book should be highly commended.

Professor E. N. Pawlowsky of the Zoological Institute in the Academy of Military Medicine at Leningrad has done a great service in publishing (Centr. Bakt. Par., I. Ref., 82: 97) a splendid critical summary of Russian parasitological literature from 1914-1923.

NOTES

Wohlfahrtia vigil a Parasite upon Rabbits.—About the first of August there was brought to me a full grown larva of the Sarcophagid, *Wohlfahrtia vigil* Walker, which had been extracted from a hole above the nose of a very young cotton-tail rabbit, with the statement that two similar larvae had been removed from the side of the neck of a second rabbit. The animals were being reared as pets and said to have been "probably not over a week old." The insect has been reported by Walker (Jour. Par., 7: 1, 1921; 9: 1, 1922) as a human parasite and has been experimentally reared here by Mr. R. C. Shannon. It should be noted that the litter of rabbits was found in Ithaca, N. Y., within 50 rods from where I have taken specimens of adult flies of this species off and on for several years. This case of parasitism upon rabbits under natural outdoor conditions is of interest as it leads one to suspect that the rodent is a natural host for this dipteron.

I take this opportunity to correct an error which was made in the paper dealing with the first instar of this species published in this JOURNAL (7: 154, 1921). The description was based upon an alcoholic specimen in which the median hook was wholly retracted, and only the tips of the oral rods showing. When the specimen was cleared and mounted in balsam the well developed median hook was readily seen. Walker (Jour. Par., 9: 1, 1922) has described the mouth parts of this instar.

O. A. JOHANNSEN.

NOTICE

The second annual meeting of the American Society of Parasitologists will be held in connection with the meeting of the American Association for the Advancement of Science in Philadelphia from December 27 to 31, 1926. Professor G. H. F. Nuttall, of Cambridge University, England, will be a guest of the Society and will deliver an address. The membership of the Society is now well over 350. The secretary will be glad to receive from members recommendations for membership of persons interested in some phase of the subject of Parasitology.

W. W. CORT, Secretary.

ALLEN JOHN SMITH

December 8, 1863—August 18, 1926